Third WHO Global Consultation on regulatory requirements for xenotransplantation clinical trials, Changsha, Hunan, China December 12-14, 2018: "The 2018 Changsha Communiqué" The 10-Year Anniversary of The International Consultation on Xenotransplantation

Hawthorne, Wayne J.; Cowan, Peter J.; Bühler, Léo H.; Yi, Shounan; Bottino, Rita; Pierson, Richard N.; Ahn, Curie; Azimzadeh, Agnes; Cozzi, Emanuele; Gianello, Pierre; Lakey, Jonathan R. T.; Luo, Minhua; Miyagawa, Shuji; Mohiuddin, Muhammad M.; Park, Chung-Gyu; Schuurman, Henk-Jan; Scobie, Linda; Sykes, Megan; Tector, Joseph; Tönjes, Ralf Reinhard; Wolf, Eckhard; Nuñez, José R.; Wang, Wei

Published in:
Xenotransplantation

DOI:
10.1111/xen.12513

Publication date:
2019

Document Version
Author accepted manuscript

MEETING REPORT
Final Draft: 04 March, 2019
Word count 19,242

Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials, Changsha, Hunan, China 12 - 14 December, 2018

"The 2018 Changsha Communiqué"

The 10 Year Anniversary of The International Consultation on Xenotransplantation

Authors;
Wayne J. Hawthorne[1], Peter J. Cowan[2], Léo H. Bühler[3], Shounan Yi[4], Rita Bottino[5], Richard N. Pierson III[6], Curie Ahn[7], Agnes Azimzadeh[6], Emanuele Cozzi[8], Pierre Gianello[9], Jonathan RT Lakey[10], Minghua Luo[11], Muhammad M Mohiuddin[12], Chung-Gyu Park[13], Henk-Jan Schuurman[14], Linda Scobie[15], Megan Sykes[16], Joseph Tector[17], Ralf Reinhard Tönjes[18], Eckhard Wolf[19], Peng Zhibin[20], Shuji Miyagawa[21], José Ramón Nuñez[22], Wei Wang[23]

Affiliations
[1] Department of Surgery, Westmead Hospital, Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney Medical School, University of Sydney, Westmead, NSW, Australia
[2] Immunology Research Centre, St Vincent's Hospital, Melbourne, Australia
[3] University Hospitals Geneva, Geneva, Switzerland
[4] Center for Transplant and Renal Research, Westmead Institute for Medical Research, University of Sydney, Australia; Transplantation and Gene Therapy Institute, Third Xiangya Hospital of Central South University, Changsha, China
[5] Allegheny Health Network – Carnegie Mellon University – Pittsburgh, PA, USA
[6] Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[7] Division of Nephrology, College of Medicine, Seoul National University, South-Korea
[8] Transplant Immunology Unit, Padua University Hospital, Padua, Italy
[9] Université Catholique de Louvain – Health Science Sector – Laboratory of Experimental Surgery and Transplantation, Brussels, Belgium
[10] Department of Surgery and Biomedical Engineering; Clinical Islet Program, University of California Irvine, USA
[11] State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
[12] Cardiac Xenotransplantation Program, University of Maryland School of Medicine, Baltimore, MD, USA
[13] Department of Microbiology and Immunology, Seoul National University College of Medicine; Department of Biomedical Sciences, Seoul National University Graduate School; Xenotransplantation Research Center, Seoul, South-Korea
[14] SchuBiomed Consultancy, Utrecht, The Netherlands
[15] Department of Biological and Biomedical Sciences, School of Health and Life Sciences, Glasgow Caledonian University, Glasgow, Scotland, UK
[16] Columbia Center for Translational Immunology, Department of Medicine, Columbia University and Columbia University Medical Center, New York, NY, USA
[17] University of Alabama Birmingham School of Medicine, Birmingham, AL, USA

[18] Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, Division of Medical Biotechnology, Section 6/4 Non-vital Tissue Preparations and Xenogeneic Cell-Therapeutics, Langen, Germany
[19] Gene Center, Ludwig Maximilian University of Munich, Munich, Germany
[20] Division of Infectious Disease, Chinese Center for Disease Control and Prevention, Beijing, China
[21]Division of Organ Transplantation, Department of Surgery, Osaka University, Osaka, Japan
[22]World Health Organization, Geneva, Switzerland

[23]Cell Transplantation and Gene Therapy Institute, Third Xiangya Hospital of Central South University, China.

Address for correspondence
Wayne Hawthorne, Department of Surgery, Westmead Hospital, Centre for Transplant and Renal Research, Westmead Institute for Medical Research, Sydney Medical School, University of Sydney, Westmead, NSW, Australia
Phone: +61 2 9893 7440
E-mail: Wayne.Hawthorne@sydney.edu.au
or
Henk-Jan Schuurman, PhD, SchuBiomed Consultancy, Utrecht, The Netherlands
Phone: 0031 6 3195 3962
Email: schubiomed@gmail.com

The World Health Organization (WHO) has a long-standing interest in xenotransplantation, which started with the publication of the "WHO Guidance on Xenogeneic Infection/Disease Surveillance and Response: A strategy for International Cooperation and Coordination" in 2001 [1]. A major milestone being resolution WHA57.18 of the 57th World Health Assembly in 2004, urging member states, amongst others, to perform xenogeneic transplantation only "when effective national regulatory control and surveillance mechanisms overseen by national health authorities are in place" [2]. This resolution followed a position report published by the International Xenotransplantation Association (IXA) on ethical aspects pointing to the requirements of adequate preclinical data, proper oversight by competent authorities, and approval by institutional bodies overseeing the ethical conduct of human research and animal welfare [3]. The WHO resolution was followed by global consultations, the first one being a Xenotransplantation Advisory Consultation in Geneva in 2005 [4], which was followed by the first WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials, published as the Changsha communiqué in 2008 [5]. In 2011 the Second WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials was held in Geneva focusing on xenotransplantation-associated infectious risk [6,7].

The major progress in xenotransplantation research and development over the past few years motivated the necessity for an update of these WHO documents, in particular the Principles and Recommendations to various parties including WHO itself, member states, and investigators. Professor Wayne J Hawthorne from Sydney, Australia took the initiative to mark the 10-year anniversary of the Changsha Communiqué with the organization of the third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials. In collaboration between WHO, IXA and the Third Xiangya Hospital of the Central South University, Changsha, Hunan, China, this meeting was held in Changsha, December 12-14, 2018.

The Central South University and The Transplantation Society served as host, with Dr Guogang Zhang, President of the Third Xiangya Hospital chairing the opening of the meeting. The WHO was the convenor and secretariat being formally represented by Professor José Ramón Nuñez. The program committee included Professor Wayne J Hawthorne (Program Chairman, Sydney, Australia), Professor Peter Cowan (Past President of IXA, Melbourne, Australia) and Professor Léo Bühler (President IXA, Geneva, Switzerland): Professor Wei Wang and Professor Shounan Yi (Central South University, Changsha) served on the local organizing committee.

There were 36 invited participants from many countries around the world including participants from Asia. A number of dignitaries also attended and provided speeches along with 20 invited international experts who presented updates in the field in two sessions during the first part of the meeting. This was followed by a session on the regulatory environment and the role of WHO and IXA in the regulation of xenotransplantation. Thereafter, the Principles and Recommendations of the Changsha Communiqué were reviewed and discussed in separate sessions by six working parties: 1) xenozoonosis; 2) regulatory; 3) biorepository; 4) transgenic pig facilities; 5) biomaterials and encapsulation; and 6) immunosuppression and tolerance induction. The Document from the Second Global Consultation (Geneva, 2011) was also included in these discussions.
After feedback from the working parties, the final session focused on the revision of the WHO documents resulting in the formulation of the draft "Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials, The 2018 Changsha Communiqué". This draft was submitted to WHO in February 2019 for approval and will then be posted on the websites of WHO, IXA and TTS, and published in Xenotransplantation. This report includes summaries of the various sessions, followed by the abstracts of invited speakers from the update sessions.

Introduction and Opening Session

The meeting was formally opened by Dr Guogang Zhang, President of the Third Xiangya Hospital, The Central South University, and Chairman of the Conference. Following his overview remarks and thanks to all participants from within China, Asia and the rest of the world he declared the meeting open.

Dr José Ramón Nuñez then provided a comprehensive introduction to the meeting, outlining the ongoing support from the WHO. In his opening speech he stated that it was his great pleasure and honor to attend on behalf of the WHO. He thanked the Hunan Authorities for hosting and supporting this important meeting, and also the local and international organizers for their great job and effort to make it happen. For WHO, it is a priority as mandated by Members States to achieve Universal Health Coverage, and under this framework it is the responsibility of WHO to give the best evidence-based response to patient's needs, from prevention to transplantation. Shortage of available organs unfortunately is a reality these days, and all efforts should be done to globally improve the quality of life and survival rates. Xenotransplantation is an open door while ethical and safety measures are followed. WHO thanked the Hunan Authorities for hosting and supporting this important meeting and also the local and international organizers forth meeting of WHO Executive Board in May 2003.

WHO's interest in xenotransplantation started following discussions of access to transplantation and its safety and ethics during the 112th meeting of WHO's Executive Board in May 2003. The World Health Assembly formalized its approach to Xenotransplantation in Resolution 57.18 on human organ and tissue transplantation adopted in May 2004: in its section related to xenotransplantation, the Director General of WHO was asked to take further action to improve communication and collaboration among health authorities in Member States; to collect data on xenotransplantation practices; to inform members of any xenogeneic infectious events; and to provide technical support in the field to Member States and report back to the Assembly.

It was clear to WHO that xenotransplantation could bridge the gap between demand for, and supply of, human organs for transplantation. In April 2005 a meeting was arranged in response to the Resolution 57.18. During this meeting which took place in Geneva, xenotransplantation was defined as the transplantation, implantation, or infusion into a human recipient of living xenogeneic cells, tissues or organs, and human bodily fluids, cells, tissues or organs that have had ex vivo contact with these living xenogeneic materials. It was recognized that animals are a potential source of high quality, readily available live organs, tissues or cells for transplantation, but that three problems needed to be overcome, i.e., inadequate physiological function, rejection of the graft and the risk of transmitting a serious and/or novel infectious disease to the human recipient. But at the same time it was highlighted that successful xenotransplantation of organs could benefit many people. Xenotransplantation of tissues and cells also offers a potential treatment of diseases such as diabetes and some degenerative disorders.

All of these concerns were clearly articulated in the Changsha Communiqué in November 2008. Key recommendations were made to WHO, to Member States and to investigators and sponsors of clinical trials using xenotransplantation products. Dr Nuñez did not go into the very well-defined recommendations of the Changsha Communiqué that are well known, but it is clear that all xenotransplantation clinical trials and procedures need to be effectively regulated and there should be no xenotransplantation in the absence of effective regulation by the government of the country. The regulatory system should be transparent, must include scientific and ethical assessment, and should involve the public.

In 2011 the Second WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials was convened, and the importance of consensus between health authorities, experts and professionals on the various safety requirements for xenotransplantation clinical practice was pointed out as was discussed in Changsha. Participants at this meeting agreed that scientific, regulatory, and legal tools, applied in the context of rigorous adherence to the Changsha Communiqué's principles and recommendations, need to be adequate to protect public safety, and that the principles and guidance contained in the Communiqué remained valid.

Since then, significant progress has been reported from preclinical xenotransplantation trials, mainly related to efficacy and infection risks, but better information on xenotransplantation trials and activities taking place in Member States is necessary to harmonize practices and facilitate collaborations.

This meeting provided further guidance, insisting in particular on the need for transparency and external assessments of protocols, of their implementation and outcomes. Transparency in the development of national policies and procedures and in the conduct of any xenotransplantation trial, including (but not limited to) the trial design is essential to ensure harmonized practices and an acceptable level of safety. Considerable effort has already gone into improving the effectiveness and to minimizing the risks. As mandated by its Member States, WHO is strongly committed to working on concrete measures that can ensure that organ donation and transplantation are always carried out in a well-regulated, safe, and ethical way.

IXA is putting these concrete measures in place, according to its mission “to promote xenotransplantation as a safe, ethical and effective therapeutic modality by: fostering the science of transplantation through promotion of ethical clinical and preclinical research; educating health care providers and society in an interactive public debate; and guiding the development of scientifically public policy in the field considering different social, ethical and legal frameworks. Dr Nuñez assured full support from WHO. The present Global Consultation was designed to discuss planned or ongoing xenotransplantation clinical activities and provide a framework for exchanges identifying needs for advice and collaborations.
It was seen as a great opportunity to review, discuss and update if needed, this important communique and to set the pathway forward based on WHO Guiding Principles.

SESSION I - Update on progress in solid organ xenotransplantation (2012-18)

Dr Wayne J. Hawthorne opened this session with an introduction of participants and outline of the program objectives. He outlined that there had been more than 40 invited participants with a total of 36 invited participants attending the meeting. From these there were a total of 25 speakers providing introduction and overview with updates of the latest innovations occurring in the xenotransplantation and regulatory fields. There were a total of 10 Chairs appointed for the various sessions of the meeting and also 6 group Rapporteurs/Chairs were elected for the comprehensive review of the Changsha Communiqué Principles and Guidance documents. The focus was to critically review;

- New technologies,
- Donor animal genetics,
- Donor source herd holding requirements,
- Changing legislative frameworks (internationally),
- Definitions and processes for submitting clinical trial applications.
- Emergence of a number of potential Xenotransplantation trials in islet cell, cornea and kidney transplantation.
- Revisiting and prioritizing essential requirements for clinical trials.

The role of the working parties was to propose changes, if required, to the guidelines and policy in order to safeguard mandatory safety and efficacy of imminent clinical trials.

Dr Hawthorne referenced the first and second WHO Global Consultation meetings, held in 2008 (Changsha [5]) and 2011 (Geneva [6]), respectively, and then presented the aims of the 3rd meeting:

- To review the current status of xenotransplantation science and practice;
- To determine whether updates to the Changsha Communiqué's guidance to WHO, member state health regulatory authorities, and study investigators and/or sponsors of xenotransplantation trials are required; and
- To discuss and refine draft consensus guidelines for infectious disease surveillance, prevention, and response appropriate to support various imminent clinical xenotransplantation trials.

After presenting the program of the present meeting, Dr Hawthorne concluded his introduction with the remark that this WHO Global Consultation has the prospect to strongly reconfirm the contribution by IXA and WHO to the regulation of xenotransplantation, and to provide a revision to WHO Mémoire on minimizing risks in xenotransplantation with a realistic potential for success in pilot clinical trials in selected patients.

SESSION I - Update on progress in solid organ xenotransplantation (2012-18)

In this introductory update and overview session five international experts provided insight into current advances in the development of technologies for solid organ xenotransplantation and the regulatory frame works that are required to work within.

Dr Muhammad Mohiuddin reviewed preclinical studies in heart xenotransplantation. He outlined progress in the model of heterotopic pig heart transplantation in nonhuman primates, with the longest survival being over 900 days: this achievement required transgenic expression of the complement regulator CD46 (membrane cofactor protein) and thrombomodulin on the background of α1,3-galactosyltransferase gene knock-out, in combination with costimulatory blockade using anti-CD40 antibody in the immunosuppressive cocktail. In long-term survivors a major contribution to rejection was anti-non Gal antibodies. He then reviewed the achievements in the orthotopic heart transplant model, with special focus on the recent *Nature* publication by the Munich group, reporting survival of 195 days (protocol study endpoint with functional graft). Among the lessons learned in this study was the need for optimal donor heart preservation before transplantation and use of anti-hypertensive drugs after transplantation, and control of graft growth by rapamycin. Dr Mohiuddin concluded with a perspective on clinically applicable drug regimens and an outlook on potential clinical conditions for which cardiac xenotransplantation may be justified. The key points that he felt necessary to move the field forward to clinical trials were;

- Clinically approved immunosuppression for clinical translation;
- Experimental evidence for the benefit of additional genes;
- Ability of multiple centers to reliably reproduce the Munich results;
- Continuation of the dialogue with regulatory agencies and commencement of a public awareness campaign.

Dr Joseph Tector gave an overview on preclinical studies in kidney and liver xenotransplantation. His presentation was based on the question of what information can be gained from a preclinical transplant model in nonhuman primates. Regarding kidney, survival data are gradually improving, with the longest survival in baboons being 260 days, and in monkeys 435 days. Interestingly, low levels of anti-pig antibody before transplantation were associated with long survival after transplantation. Also, observations in the kidneys that failed include features of glomerular and thrombotic microangiopathy, basement membrane

thickening and depositions of immunoglobulins and complement. This fits with early antibody-mediated rejection as a major cause of graft failure in the long term. Studies on donor-recipient crossmatches revealed that there is no negative crossmatch in the pig-to-nonhuman primate model, and that the best possible combination is a donor with a knock-out of the β1,4-N-acetyl-galactosaminyltransferase2 gene and a rhesus macaque recipient: but, essentially the pig-to-nonhuman primate model does not allow the assessment of a negative crossmatch on graft outcome. Hence, the outlook is to generate new pigs with a negative crossmatch. Regarding liver transplantation, Dr Tector presented data on meaningful life-supporting function of a porcine liver in a nonhuman primate, but survival is low and more preclinical studies are needed using new genetic engineering strategies before considering a phase transition to clinical trials.

Dr Agnes Azimzadeh provided a summary on preclinical studies in lung xenotransplantation. After an introduction on rejection mechanisms, results in an ex vivo lung perfusion model were reviewed. This model enables the assessment of the role of coagulation and inflammation, with a beneficial effect of inhibitors in the clotting reaction, inflammatory reactions and platelet aggregation. Regarding the coagulation cascade this was illustrated for the efficacy of tissue factor pathway inhibitor, thrombomodulin and endothelial protein C receptor. The model was used in evaluation of a large series of lungs from genetically modified pigs. In the model of in vivo lung transplantation into nonhuman primates, an increase in survival during the last years was noted, but survival rates remain low (up to 30 days). Dr Azimzadeh mentioned that injury of the vascular barrier function (leading to interstitial and airway edema) was among the significant remaining challenges.

Dr Megan Sykes reviewed the current technologies and studies in the development of tolerance and use of regulatory T-cells. The major tolerance approaches remain focused on mixed chimerism and thymic transplantation. In rat-to-mouse models mixed chimerism prepared in combination with non-myeloablative conditioning can affect both T-cell mediated and non-Gal antibody-mediated rejection, and in addition induces tolerance of NK cells. These achievements in the rat-to-mouse model apply also to the pig-to-human combination, with evidence that central T-cell tolerance can be achieved through mixed pig/human hematopoietic chimerism. The possibility to tolerize B cells producing anti-pig antibodies may represent an alternative to generating knock-out pigs being deficient in carbohydrate epitopes. However, mixed chimerism is short-lived in the pig-to-baboon condition, with a major role for macrophages. To this end, transgenesis of CD47 (integrin associated protein) was introduced in pigs, resulting in increased porcine hematopoietic chimerism in baboons and prolonged porcine skin graft survival. This chimerism and skin graft survival was further prolonged when regulatory T-cells were added to the protocol. The beneficial effect on tolerance using CD47-transgenic pig donors was also evident in other transplantation models. The efficacy of porcine thymus transplantation was first demonstrated in pig skin transplantation in mice, and further developed for vascularized thymic transplants in pig-to-baboon kidney transplantation using α1,3-galactosyltransferase gene knock-out pigs. In this model there was evidence for T-cell tolerance in in vitro assays. Interestingly, Dr Sykes mentioned that expanding the work in this transplant model revealed that CD47 transgene expression on glomerular cells appeared to minimize the development of proteinuria and nephrotic syndrome.

Dr Henk-Jan Schuurman gave an overview on regulatory aspects of xenotransplantation. The focus of regulatory agencies is on the full flow chart, i.e., starting with the source pig herd and donor animal, continuing with the subsequent processing or manufacturing of the product, and ending with the monitoring of the patient. Reference was made to recommendations in earlier WHO consultation meetings. The microbiological status of the source herd and donor animals, in particular, with regard to the different lists in the literature on excluded pathogens, and the requirements for barrier facilities. Dr Schuurman noted that these requirements may be less for cell therapy products than for solid organs because of

the time period in cell product manufacturing, with supporting data for a pig islet product. There is a broad regulation of cell therapy products, in contrast to that of organs: this is explained by the fact that a human organ in clinical transplantation is not a medicinal product. A proposal for release testing of a porcine xeno-organ is in preparation. Archiving and documentation is required for clinical application of xenotransplantation products, and it is proposed to implement this in collaboration between public health institutes and trial sponsors. Regulations of xenotransplantation are increasingly implemented worldwide, with major developments in Asian countries. Since the field of xenotransplantation is moving fast, Dr Schuurman advocated that there should be a continued dialogue between regulatory agencies and the science/technology arena, to reach agreement in complex issues.

SESSION II a -
Update on progress in cell and tissue xenotransplantation (2012-18)

Five international experts were invited to provide an overview of the most recent developments in pre-clinical studies involving porcine cells/tissues.

The consensus of the IXA is for rigorous pre-clinical studies focusing on the nonhuman primate model as a requirement prior to consideration of clinical applications. Successes in pre-clinical studies have led us to seriously contemplate clinical trials, emphasizing the importance of regulatory and ethical frameworks. In this session;

Dr Rita Bottino spoke on the topic of free islet xenotransplantation. Recently, islets from wild type and genetically engineered pig donors have both resulted in successful treatment of nonhuman primate diabetic recipients. Free islets are targets of innate and adaptive immunity, thus far requiring high doses of islets and non-clinically applicable immunosuppressive drugs. Still, multiple groups worldwide have reported islet graft function and insulin independence, most notably the Seoul National University, which has reported insulin independence for more than 900 days. The further advancement of genetically modified pig donors, possibly using more efficient methods of genetic manipulation such as CRISPR/Cas 9 and the development of effective immunosuppression may lead to more patient friendly anti-rejection protocols that protect the xenograft without increasing the risk of over-treatment.

Dr Jonathan Lakey outlined advances in encapsulated islet/device transplantation as a treatment for diabetes. Encapsulation represents the promise of islet transplantation without immunosuppression. The general concept is based on a biocompatible matrix surrounding the islet cells that allows diffusion of oxygen and nutrients while preventing large immune molecules from reaching the cells, thus avoiding the host immune response. Synthetic agents and naturally occurring hydrogels have been used in the encapsulation process with varying degrees of success. Alginate encapsulation is highly popular due to its biocompatibility, stability, ease of use, and low cost. However, variations in alginate production and purification have led to issues with endotoxin content. Moreover, lack of knowledge regarding optimal transplantation sites and optimal donor pig strains and age has also contributed to inconsistent results in pre-clinical as well as in a few pilot clinical trials. Efforts to improve permeability and strength of the capsule have often resulted in a greater host biologic response to the islet transplant. Recent significant technical improvements and the contribution of bioengineering science have shown that it is possible to produce more efficient encapsulation devices.

Dr Chung-Gyu Park presented an overview of the preclinical data on porcine corneal xenotransplantation in non-human primates carried out at the Seoul National University in South Korea. Both the techniques as well as the effects of different immunosuppressive protocols were presented. Moreover, Dr. Park focused on a proposed clinical trial of cornea xenotransplantation sponsored by his Institution and designed in response to the need for a

suitable supply of corneas to treat patients with corneal blindness. Unfortunately, the current supply of human donor corneas does not meet the demand in many areas of the world. The proposed trial is designed in accordance with the international consensus established by the IXA and WHO to ensure safety, efficacy and transparency. The original protocol developed for the trial was reviewed by a team of international multidisciplinary experts and modified in response to their comments. Two candidates will be enrolled in the corneal study and only after evaluation of the initial results will the project be allowed to proceed.
As this will be one of the first trials of this kind, setting the precedent for the international community, particular attention will be paid to the safety of the procedure. To this aim, the sponsors and SNU have requested as a pre-requisite that national regulatory agencies oversee and implement all necessary regulations to provide best practice in regard to the protection of the general population from potential unexpected events arising from the proposed study.

Dr Leo Buhler spoke on the topic of hepatocyte xenotransplantation. He provided an update of preclinical trials stating there has been one conducted at the University of Geneva where high volumes of alginate microencapsulated porcine hepatocytes were transplanted into the peritoneum of baboons immediately after induction of acute liver failure. The data demonstrated that the microencapsulated hepatocytes from miniature swine were able to provide temporary functional support for baboons with fulminant liver failure, in the absence of pharmacological immunosuppression. There are plans to establish a DPF pig facility and GMP-grade laboratory to repeat the preclinical study with a larger number of animals. Additionaly, mesenchymal stromal cells will be co-transplanted with the hepatocytes to potentially improve graft viability. The Swiss Federal Regulatory Agency for Drug Administration has been contacted to establish a road map for initiation of a clinical trial.

Dr Megan Sykes presented an overview on transition to phase I/II clinical trials.
The Basic Ethical principles of Respect for Persons, Beneficience and Justice predicated by the Belmont Report (1979) are the cornerstone of all trials, as the moral rules to follow when addressing specific challenges in xenotransplantation. Adherence to all applicable regulatory agency guidelines and international standards including the Changsha Communiqué remain fundamental requirements for the design of sound clinical trials.
Important requirements should be observed in order to move forward with a porcine organ or tissue trial, including a patient population for whom there are no good alternatives (bridge vs destination therapy), justified use of immunosuppression, compelling nonhuman primate data, likelihood of benefit to the individual patient, and low risk to the community. To this aims, source animals should meet the highest biosecurity and production standards including quality control assessment of the gene modifications.
Trials of kidney or heart xenotransplantation should be considered exclusively for patients with hyper sensitization against human antigens, who may not be able to receive a human organ. Treatment of fulminant liver failure via liver transplantation as a bridge to recovery or transplant may be contemplated, most especially in view of recent improvements in treating thrombocytopenia.
Islet xenotransplantaion, an alternative to insulin therapy, may represent a more challenging arena to justify immunosuppression than with heart or kidney recipients. Treatment of severe conditions such as hypoglycemia unawareness should be evaluated and measured against the risk of general immunosuppression. Protocols aimed at tolerizing the recipient towards the donor, thus reducing the need for systemic immunosuppression may significantly and positively impact the focus for islet xenotransplatation. Careful consideration should be given to combining islets with a kidney transplant in patients with diabetes and renal failure.
The unique exigencies of xenotransplantation require consideration of both individual persons and the whole of society. Our commitment to the basic ethical principles of justice, beneficence, and “do no harm” remains firm. Analysis of risk and reward shows that certain tightly regulated clinical trials are already justified while envisioned scientific progress will continue to alter the risk/reward balance in favor of clinical trials xenotransplantation.

SESSION II b - Update on GM pigs & facilities, proposed clinical trials, and microbiological testing

Dr Ralf Tönjes chaired this afternoon session which encompassed four sections.

First, **Dr Eckhard Wolf** gave a state-of-the-art overview on genetically-modified pigs. His presentation focused on methods for pig genetic engineering including the use of RNA-guided nucleases, i.e., gene editing using CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR associated protein 9), and somatic cell nuclear transfer. This includes the use of polycistronic vectors in transgene 'combineering', and the value of nanopore sequencing in the characterization of the transgene locus. This was followed by a review of various genetic modifications generated in pigs, including the inactivation of PERV, and functional results achieved in transplantation. Besides gene editing and immunosuppression, Eckhard focused on physiology, e.g., the relevance of organ growth leading to the generation of growth hormone receptor-deficient pigs. The presentation ended with examples of specific genetic modification for islet transplantation addressing the instant blood-mediated inflammatory rejection (IBMIR), islet immune protection, and engineering to enhance insulin secretion. Dr Wolf concluded that genetic engineering of donor animals presents unique opportunities in xenotransplantation and has accelerated the progress in the field with remarkable achievements in long-term graft survival rates. The type of cell/tissue/organ determines the combination of genetic modifications. The cellular localization and level of transgene expression is crucial with respect to functionality and avoiding adverse side effects of a specific genomic modification.

The second session was a review of the pig barrier facilities around the globe;

Dr Curie Ahn gave an overview of four pig barrier facilities in Korea.

- The Biomedical Center for Animal Resources with a capacity for 50 miniature pigs, originally Minnesota miniature pigs imported from the Chicago Medical School in the USA, and now described as SNU (Seoul National University) miniature pigs. The animals are housed under designated pathogen-free (DPF) conditions. This facility has operated since 2002, and was described in detail regarding its infrastructure, including rooms for Caesarian delivery and isolator units, pig housing and herd monitoring. As an example, Dr Ahn mentioned the first DPF pigs expressing the complement regulator CD55 (human decay-accelerating factor) generated by somatic cell nuclear transfer.
- The Designed Animal Resources Center with a capacity for 150 miniature pigs, with focus on genetically-engineered animals. The facility, in operation since 2014, encompasses different zones for holding miniature pigs, and has in addition a zone for mouse holding and a zone for holding nonhuman primates. The facility is equipped with all tools in advanced animal handling including the production of genetically-engineered pigs (CRISPR/Cas9 gene editing and somatic cell nuclear transfer). Dr Ahn illustrated this with various genetically-engineered pigs for use as donor in cell (islet) and organ xenotransplantation.
- The Optipharm facility, which accommodates 350 miniature pigs under SPF conditions in two zones. The facility is in operation since 2014. The microbiologic monitoring was presented, based on which animals can be used as xenotransplantation donor. This was illustrated by the eradication of porcine circovirus 2 from the facility. The facility encompasses a diagnostic laboratory, the Optipharm Evaluation Center, for testing of infectious agents and diagnosis of animal diseases.
- The National Institute of Animal Science with a capacity to house 20 pigs under SPF conditions and 80 pigs under DPF conditions. The facility, in operation since 2015,

was described regarding its infrastructure and equipment for generating genetically-modified animals. Dr Ahn illustrated this for the first α1,3-galactosyltransferase gene knock-out miniature SNU pig generated in Korea, and the subsequent generation of animals transgenic for CD46 and thrombomodulin or CD73 (ecto-5′-nucleotidase). Amongst others, these animals serve as donor for cornea transplantation.

Dr Wei Wang reviewed the pig barrier facilities in China. One of the major projects undertaken by the Central South University, Changsha group was the search for an ideal pig donor, the basis of an extensive search was for an animal that was negative for porcine endogenous retrovirus (PERV) type C, which is considered the driver for pig-to-human PERV transmission. After its identification, a pig line negative for PERV-C was established, and inbred for 22 generations. A barrier facility for DPF pigs was constructed in Changsha and started operations in 2012. This facility has the infrastructure and equipment to breed and maintain animals in DPF conditions in compliance with the regulations in China. Dr Wang illustrated this with the list of pathogens excluded from the herd with stringent ongoing maintenance and conditions such as specialized feeding. The animals in this facility were used as donors in a pig islet clinical trial overseen by the respective China governmental agency: the design of this trial was outlined in detail regarding the biosafety monitoring. This trial included the implementation of a database for clinical trials, and archiving of medical records. The trial was also registered at ClinicalTrials.gov (NCT03162237). There were no cross-species infections documented in this trial and importantly no cross-species transmission of PERV.
Dr Eckhard Wolf gave an overview on pig barrier facilities in Europe, including a facility in Italy managed by Cecare Galli (Avantea) and one in Munich which accommodates pigs under SPF conditions. Pigs in this facility are negative for porcine cytomegalovirus, achieved by the immediate separation of newborns from their sows at birth, and immediate removal of CMV-positive sows from the herd. These pigs are used in subsequent genetic engineering projects, under detailed hygienic monitoring (Vaxxinova veterinary prevention strategies).

Dr Agnes Azimzadeh provided a summary of pig barrier facilities in the USA. The regulatory landscape in the USA was introduced, with focus on guidance's from the Public Health Service and the FDA. Elements for donor pigs include a closed herd in adequate barrier facilities, including a health screening program and monitoring for infectious agents. Regarding infectious monitoring reference was made to the pathogen list published at the WHO Global Consultation Meeting in Geneva, 2012, and a reduced list proposed by Dr Jay Fishman: this list was subsequently adapted based on experience with immunosuppressed human allotransplant recipients and experience in pig-to-nonhuman primate xenotransplantation studies. Two animal facilities were described; the barrier facility run by Spring Point Project, and the barrier facility run by the University of Alabama. The Spring Point facility recently published details of its operation including the monitoring regimen for infectious agents. The Alabama facility is presently used for generation of genetically-engineered pigs for research projects. In a futuristic approach, the basic design of a large-scale animal facility proposed by United Therapeutics (a US biotechnology company based in Maryland) was presented. This facility has a modular design to facilitate a barrier function and to provide expansion based on demand.

The third section of this session was a review of proposed clinical trials.

Dr Chung-Gyu Park described the forth coming proposed Korean clinical cornea and islet trials, using fresh porcine corneas and naked adult porcine islets. These trials have been designed following extensive consultation and focus on the global consensus (IXA and WHO) published in Xenotransplantation. Key items in the design are (1) the source pig; (2) the harvest and manufacturing of the product; (3) support data on efficacy and safety from preclinical transplantation in nonhuman primates; (4) outline of recipient monitoring and management; and (5) patient selection criteria. Donor animals are the SNU miniature pig

described above. The protocols were recently discussed in a meeting with the Ethics Committee of IXA, with input from The Transplantation Society (TTS). The cornea xenotransplantation is foreseen in adults who have corneal opacity and bilaterally legal blindness (excluding keratoconus), with primary endpoint in safety follow-up for 2 years and efficacy follow-up for 1 year. The islet xenotransplantation is foreseen in adults with diabetes for >5 years, with a first trial in 2 patients at a dose of 15,000 islet equivalents (IEQ) per kg body weight and a primary endpoint in a follow-up period of 16 weeks, and a subsequent trial being a dose escalation to determine the optimal mass of islets.

Dr Joseph Tector then outlined a provisional clinical trial of porcine kidney xenotransplantation. The basic paradigm for the trial is based on that in human allotransplantation, i.e., a donor-recipient crossmatch; for xenotransplantation the elimination of xenoantigens is added. The question underlying a trial is whether a patient with end-stage renal disease can be subjected to pig kidney xenotransplantation with clinically approved immunosuppression and a one-year dialysis-free follow-up. Xenoantigens to be deleted are galactose-α-1,3-galactose (Gal), *N*-Glycolylneuraminic acid (Neu5Gc), and β-1,4-N-acetyl-galactosaminyltransferase 2 (B4GALNT2). Dr Tector proposed a modification of the crossmatch assay used in clinical allotransplantation for application in xenotransplantation, because a careful identification of a crossmatch-negative patient is crucial. I this way, patients that are highly sensitized to HLA Class I could be unsuitable as recipient of a human kidney, but on the other hand have a suitable crossmatch and be eligible to receive a porcine kidney. The selection of a potential recipient needs also careful attention including management of immunosuppression, and eligibility for a human transplant. A tiered approach is foreseen, with first explorations in small numbers of patients: it is realized that preclinical modeling lacks translational value regarding items like crossmatch. Dr Tector proposed that trial endpoints focus on a comparison with dialysis and not on a comparison with a human allograft, and also freedom of antibody-mediated rejection: these comments fit with the interpretation of preclinical modeling (see Dr Tector's presentation above).

The final section of this lengthy afternoon session was a review of prevention of infectious risk associated with xenotransplantation and current progress presented by **Prof Linda Scobie**. Initial reference was made to Principles 2 and 3 of the Changsha Communiqué, regulatory guidances/guidelines, and a recent thematic issue on safety in the journal Xenotransplantation. The presentation focused on the areas of pathogen surveillance and diagnostics (in both donor and recipient) and the relevance to using organs and/or cells in clinical trials.

First, exogenous pathogen testing in the donor animal was described, with focus on lack of validated commercial diagnostic assays. The porcine islet macrobead biosafety program proposed by Rogosin, USA, was given as an example of good practice for surveillance of donor and product. This was based on a cellular xenotransplant. Along with this data, evidence was provided in the evaluation of islets from pig donors in a commercial high health swine herd from Belgium. This study demonstrated that exogenous pathogens present in the animal could not be found in the islet cells. In addition, the evaluation of PERV, showed a reduced expression in (neonatal) porcine islets and the absence of a clear correlation regarding PERV expression between blood cells and pancreatic islets. Relevant was the observation that an alginate encapsulation product can prevent PERV transmission out of encapsulated islets.

This would suggest the possibility that infectious agents in the donor animal might not be considered a risk factor if these agents are not present in the final xenotransplantation product. Indeed, islets as a product may have significantly reduced risks compared to organs; this now appears to be proven questioning the need for the extensive screening proposed previously.

Concerning the risk posed by PERV, Prof Scobie reviewed arguments that this risk is considered minimal, i.e., transmission to human cells has only been shown in vitro under specific conditions, and transmission in vivo has not been found including data from clinical

trials. Also, Prof Scobie emphasized the possibility to base an estimated risk in xenotransplantation on the results in allotransplantation as proposed earlier by Dr Jay Fishman. Overall, the need for validated commercial diagnostics, consensus protocols and reporting trials is still there. The presentation concluded with reference to a clinical trial with viable skin from miniature swine that was recently approved by the FDA and is proposed to begin 2019 (sponsor Xenotherapeutics, identifier NCT03695939 in ClinicalTrials.gov)

Dr Henk-Schuurman chaired a short discussion sessions I and II. This discussion focused on the benefit to balance the risk when a xenotransplantation product is clinically applied. A number of key opinion leaders in the audience were asked for their views, as a prelude to the discussion in subsequent sessions III-V. In general, the consensus was that the progress in preclinical efficacy of porcine kidney and heart transplantation in nonhuman primates, combined with better insights into the risk, in particular the microbial risk, has resulted in an increased window between anticipated efficacy and safety in the clinical setting. As an example, the advances in orthotopic heart transplantation in baboons presented by the Munich group were mentioned.

SESSION III a - The regulatory environment for xenotransplantation clinical trials

This morning session, chaired by **Dr Megan Sykes**, provided in-depth overview of the regulatory environment, in particular the role of individual agencies in relation to the planning and conduct of xenotransplantation and clinical trials.

Dr Judith Arcidiacono (FDA) presented the FDA guidelines/policy to undertake clinical trials of xenotransplantation. After an introduction in which xenotransplantation products were defined and the revised FDA Guidance was mentioned, the FDA Centers involved in oversight were introduced. The risks of xenotransplantation products were presented in detail, and also the four target items in regulatory considerations, i.e., the source herd, the source animal, the processing and manufacture of the product and the monitoring of patients. This was further specified for the three types of products, i.e., whole organs, cells and tissues, and combination products (cells and device). The action items and interactions with FDA during the different phases of product development, i.e., preclinical, clinical, and marketing, were presented. Special attention was given to possible risks associated with genetically-altered source animals, with requirements for an Investigational New Animal Drug (INAD) and New Animal Drug Application (NADA). Dr Arcidiacono concluded this comprehensive overview with a summary of new references in the 2016 Guidance, a list of FDA Guidance documents, and contact information.

Dr Jianguo Xu described the infectious disease surveillance and control system in China. This included the notifiable disease reporting system for 39 diseases in three different categories. This system is internet based centered around a data center (Chinese Center of Disease Control). An enhanced surveillance has been introduced for 27 selected diseases and 4 vectors (rat, mosquito, fly, cockroach). Also, a public health emergency reporting system has been established. Dr Xu finished the presentation with an outline of the strategy for different groups of diseases, and data on morbity and mortality trends of infectious diseases during the last decades.

Dr Minhua Luo described the system for monitoring and control of viral infections in xenotransplantation recipients in China. This centered at the Wuhan Institute of Virology of the Chinese Academy of Sciences, and the National Biosafety Laboratory. The activities were introduced by two cases of lymphocytic choriomeningitis virus infection in renal allotransplant recipients. The need for biosafety surveillance was recognized during the 2008 Changsha meeting [5] and the pig-to-human xenotransplantation summit in Changsha held in 2012 (published in Xenotransplantation 2012; 19: 327-328). Dr Luo presented an overview of

PERV, in relation to Human Endogenous Retrovirus (HERV), and introduced a sensitive high-throughput screening for virus infection surveillance. Thereafter an overview of human cytomegalovirus in transplantation and immunocompromised individuals was presented, ending with the introduction of a novel antiviral drug, Letermovir, which is now in a late stage of development.

Dr Ralf Tönjes introduced the EU guidelines and policy to undertake clinical trials. Clinical trials in Europe are centered around Regulation (EU) No 536/2014. This regulation comprises 16 chapters. Out of these, the chapter on the authorization procedure for a clinical trial was presented in detail: this included the timelines and activities between document submission, document assessments and assessment reports, responses regarding the document and the decision on approval of the trial. Reference was made to a publication presenting the flow chart of this regulation in Nat Biotech 2016; 34: 231-233. Dr Tönjes then described the Medicinal Products Act in Germany, and the procedure in application for a clinical trial developed by the Paul Ehrlich Institute: the folder structure of the documentation was explained. A special folder is included that deals with xenogenic products. Dr Tönjes finished his presentation with the introduction of the EU Guideline on Xenogeneic Cell-based Medicinal Products issued by the European Medicines Agency in 2009, and the EU regulation 1394/2007 on Advanced Therapy Medicinal Products, which are key documents in clinical development of xenogeneic cell-based therapy.

SESSION III b - The role of IXA and WHO in the regulation of xenotransplantation

Dr Emanuele Cozzi chaired this session, which provided insight to the continued strengths in the relationships between the IXA and the WHO in ensuring the appropriate guidance is provided in relation to potential xenotransplantation clinical trials.

Dr Robin Pierson presented a summary of the IXA-FDA meetings in Boston (2016) and Baltimore (2017), and the role of the IXA Ethics Committee, which he chairs. The meeting in Boston was held during the American Transplant Congress and was attended by IXA Council members and representatives from the FDA. A number of regulatory topics proposed for re-consideration which were discussed, among which the relevance of PERV as a pathogen for humans, the requirement of 50 years in archiving materials from xenotransplantation recipients, and the classification of genetically-engineered pigs. Also, some points that are specifically related to islets were part of the discussion. As a follow-up to this meeting, a joint FDA-IXA symposium was held preceding the IXA congress in Baltimore in 2017 (published in Xenotransplantation 2017; 24: e12365): during this symposium xenotransplantation experts presented overviews on recent scientific advances in xenotransplantation, infectious safety aspects and risk-benefit analysis, and FDA representatives addressed various aspects of regulatory considerations. Among the outcomes of this symposium were (1) the conclusion that progress in the field has rendered the risk/benefit ratio more favorable for both the individual study subject and for society, and (2) the confirmation of the shared goal of FDA and IXA in advancing and protecting public health through the development of safe, effective xenotransplantation products. Dr Pierson finished his presentation by reminding attendees of the mission of IXA and emphasizing the role of the IXA Ethics Committee: a number of activities of the Ethics Committee since its introduction in 2003 [3] were presented.

SESSION IV - Review of Changsha Communiqué Principles and Guidance

Dr Emanuele Cozzi presented his views on how we have moved on since the Recommendations of the 2nd WHO Global Consultation (Geneva 2011). He started with a summary of the recommendations and addressed a number of items for revision. Dr Cozzi highlighted a number of points presented earlier in the meeting, in introducing the following points for discussion (which were further discussed in working groups described below):

1. *Xenozoonosis*: there are multiple lists of infectious agents to be excluded from the donor pig and xenotransplantation product: also, the donor animal and xenotransplantation product can differ in presence of infectious agents. Finally, there are new developments in PERV monitoring.
2. *Regulatory aspects*: new or updated documents have been issued in Europe and USA, and there are relevant developments in establishing regulatory networks in Asian countries.
3. *Biorepository*: there is ongoing discussion about materials to be archived, storage location, responsibility and financial support, and time of storage.
4. *Pig facilities*: barrier conditions have been defined, but there is a central question about the definition of 'clean' pigs.
5. *Biomaterials and encapsulation*: there are a number of developments and innovations in islet encapsulation strategies, aiming to keep islets alive, prevent a destructive host response and assure practical surgical implantation.
6. *Immunosuppression and tolerance induction*: there is the ongoing discussion (1) whether efficacy and safety should always be validated in a nonhuman primate model; (2) whether non-life-saving xenotransplantation products like kidney or cornea should be applied as new, innovative and possibly life-threatening regimens; (3) whether any new drug (even not approved) can be considered in combination with a xenotransplantation product; and (4) on the translational value of efficacious strategies in an animal model.

Dr Wayne Hawthorne established working groups to revisit the guidelines post-Geneva. Dr Hawthorne outlined the process to achieve the second and third aims of the meeting, i.e., to assess whether updates to recommendations from previous WHO meetings were required; and to discuss and refine draft consensus amendments for infectious disease surveillance, prevention, and response, appropriate to support various imminent clinical xenotransplantation trials.

To this end, participants were divided into six working parties in relation to their particular areas of expertise, and a Chairperson and Rapporteur were voted as representatives for each of the groups;

1. Xenozoonosis
2. Regulatory
3. Biorepository
4. Transgenic pig Facilities
5. Biomaterials and Encapsulation
6. Immunosuppression and Tolerance Induction

The task given to the defined groups was to review the guidelines and policies, in particular the Recommendations to Undertake Clinical Trials, in relation to the previous WHO Global Consultation Meetings (the Changsha Communiqué of 2008 [5], and the Geneva meeting of 2011 [6]), and to propose any necessary amendments.

After discussion in each working party, the proposed amendments were presented and discussed in a plenary session. This was followed by further refinement of the individual points raised. At the end of the meeting these amendments were to be finalized and phrased as a proposed change in the respective recommendations to WHO, Member State health regulatory authorities, and study investigators and/or sponsors of xenotransplantation trials.

SESSION V - Summaries from Working Parties
Main summaries regarding the Principles in the Changsha Communiqué

The working parties agreed unanimously on several modifications and amendments to the original recommendations in order to reflect the developments that have occurred since the

previous WHO Global Consultation Meetings.

The Changsha Communiqué begins with a list of 10 Principles that guide and inform the WHO position on regulatory requirements for xenotransplantation clinical trials. As we rapidly approach the possibility of clinical trials conducted under WHO guidance, it becomes a practical matter to define the scope of xenotransplantation as it relates to these guidelines.

Principle 1 has, therefore, been amended to define xenotransplantation based on the definition of the US FDA. Thus, xenotransplantation is considered as any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues, or organs from a nonhuman animal source, or (b) human body fluids, cells, tissues or organs that have had ex vivo contact with live nonhuman animal cells, tissues or organs. This specifically excludes the transplantation or implementation of acellular animal tissues, (described as “xenograft” in the revised 2016 FDA Guidance, which are beyond the intended scope of the document).

In order to better reflect the advanced state of preclinical xenotransplantation, Principle 1 has been amended to read that xenotransplantation, rather than “successful” xenotransplantation, has the potential to treat a wide range of serious diseases.

Principle 4 discusses the importance for clinical trials to be effectively regulated by the relevant governmental authorities and has been amended to emphasize the importance of monitoring post-market entry after regulatory approval of a safe, effective xenotransplantation product.

Principle 7 focuses on the long-term storage of animal and patient samples and records, even to the extent of providing for succession in the event that the original proposers of the clinical trial are unable to continue. It has been revised to emphasize that succession should also be assured for surveillance of the patients in addition to samples and records.
To highlight the advances in xenotransplantation and the likelihood of clinical trials, the language of Principle 10 has been modified to indicate that equitable access should be given to “effective xenotransplantation therapies.”

SESSION VI - Summary and Revision to WHO Mémoire on Minimizing Risks in Xenotransplantation

The third and final day commenced with **Dr Peter Cowan** revisiting and prioritizing essential requirements for clinical trials followed by **Dr Jose Nuñez** outlining the revision to the WHO Mémoire on minimizing risks in xenotransplantation and its relationship to the Changsha Communiqué. During these last sessions Dr Nuñez also helped to guide the group in its final revisions and summation of the Communiqué so that it was able to incorporate the essence of the original 2008 Communiqué and the 2011 updates.

This final session was the practical summation of the previous two days’ work specifically the incorporation of the revisions, modifications and additions undertaken to the original 2008 Communiqué and the 2011 update document. In total more than 100 changes reflecting the developments that have occurred since the previous WHO Global Consultation Meeting were agreed upon by all participants and written into the original 2008 Changsha Communiqué.

Dr Richard Pierson provided a short overview of the way forward and next steps to be taken to finalize the document from the meeting, and all in attendance agreed that there should be regular review and updating of the Communiqué. **Dr Agnes Azimzadeh** (IXA President Elect) gave a final conclusion and summary and thanked all participants on behalf of the IXA for their support and attendance at such an important meeting.

The meeting was closed by the Program Chair **Dr Wayne Hawthorne** and the Local Organising Committee Chair **Dr Wei Wang**, who thanked all those who had helped in the organisation and running of the meeting to make it such an overwhelming success. They also praised the meeting sponsors including the IXA and the TTS, whose support in planning and organisation was acknowledged. Special thanks was also given to the WHO for once again convening this important global meeting, and to the Third Xiangya Hospital and the Central South University of Hunan Province, China for their generous sponsorship, without which the meeting could not have taken place.

The draft amended Principles and Recommendations were finalized shortly after the meeting, and were submitted to WHO in February 2019 for approval and publication. After approval, this document will be published on the website of WHO and TTS (IXA website), and in *Xenotransplantation*. Below is the summary of the modifications to the recommendations.

Main summaries regarding the Recommendations in the Changsha Communiqué

Key recommendations follow the Principles established in the first section of the Changsha Communiqué. Language was modified throughout the document from “must” to “should”, in order to better reflect the purpose of this document as a series of guidelines without legal authority.

Recommendation 4 to WHO emphasizes the role of WHO in promoting public awareness of both the benefits of xenotransplantation, as well as the awareness of dangers associated with unregulated xenotransplantation. Reflecting the developments in genetic engineering of source animals since the Global Consultations in 2008 and 2011, WHO has charged itself to promote the enhanced opportunity for potential clinical trials provided by the use of animals.

The majority of recommendations, however, have been directed toward the investigators and sponsors of clinical trials using xenotransplantation products. Several amendments or modifications highlight the importance of genetically-engineered source animals including Recommendation 1 to investigators which has been updated from “specific” to “designated” pathogen-free animals to reflect current understanding of the conditions necessary to utilize specially-bred animals for xenotransplantation.

The new Recommendation 2 to investigators indicates that investigators should develop quality control measures and standards for genetically-modified pigs to ensure the desired phenotype and function of the xenotransplantation product. Recommendation 8 to investigators parallels the above recommendation to WHO in promoting public awareness of xenotransplantation, which serves to underscore the importance placed on regulation of all aspects of xenotransplantation in order to provide optimal safety and efficacy.

Recommendation 4 to investigators discusses the importance of utilizing reproducible preclinical data, usually from testing in nonhuman primates, as the basis of designing a clinical trial. It has been updated to point out that preclinical studies should be modeled as closely as possible to clinical regimens in order to yield more relevant results.

Recommendation 10 to investigators encourages the storage of appropriate specimens from patients and source animals in accordance with national regulatory guidelines and standards in quality management, and this includes a provision for stewardship of all records, data and archived samples.

A new Recommendation 10 to investigators states that devices and biomaterials should

comply with current international standards that were not in place at the time of the original Changsha Communiqué.

Finally, it was unanimously approved by the participants in the present meeting to explore appropriate changes or updates to the Geneva 2011 WHO Global Consultation document.

Conclusion

This third WHO Global Consultation Meeting proved to be very successful and productive in many aspects. Clinical application of xenotransplantation has been pursued for decades (or even centuries), but implementation that is accepted in regulatory clinical practice is of more recent date. Initiatives in this respect by respective societies and institutions are illustrated by the founding of IXA in 1996, the first publication by regulatory agencies (US PHS Guideline published in 2001), and the WHO resolution 57.18 in 2004. The continued need for close interaction between innovative science and technology, regulatory affairs, and implementation at the global level, is illustrated by the outcome of the present 2018 Changsha WHO Global Consultation Meeting. Representatives from all disciplines involved came together to discuss progress and innovations in the field, mirror this progress with respect to regulatory oversight, and foster this at the global level under the umbrella of WHO. The need for such meetings is evidenced by innovations in the field and resulting amendments in regulatory oversight and recommendations in the WHO Mémoire to synchronize developments between different countries across the globe. The perspective of this meeting was to reinforce the basis of potential success in clinical trials in selected patients.

Acknowledgements

Special thanks are to **Dr Judith (Horvath) Arcidiacono** from the Office of Tissues and Advanced Therapies, Center for Biologics Evaluation and Research, US Food and Drug Administration, who attended the meeting on behalf of the FDA and presented the FDA guidelines/policy to undertake clinical trials. Besides giving this presentation in session III, Judith provided invaluable advice and guidance as part of the committee in its revisions to the Communiqué.

References

1. World Health Organization, Department of Communicable Disease Surveillance and Response. WHO Guidance on xenogeneic infection/disease surveillance and response: a strategy for international cooperation and coordination. Document WHO/CDS/CSR/EPH/2001.2, 2001. http://whqlibdoc.who.int/hq/2001/WHO_CDS_CSR_EPH_2001.2.pdf. Accessed January 1, 2019.
2. World Health Organization. Fifty-seventh World Health Assembly, Agenda Item 12.14, May 22, 2004. Resolution 57.18. http://www.who.int/gb/ebwha/pdf_files/WHA57/A57_R18-en.pdf. Accessed January 1, 2019.
3. Sykes, M, D'Apice A, Sandrin M. Position paper of the Ethics Committee of the International Xenotransplantation Association. Xenotransplantation 2003; 10: 194-203.
4. World Health Organization. Statement from the Xenotransplantation Advisory Consultation, Geneva, 18-20 April 2005. https://www.who.int/transplantation/XenoEnglish.pdf. Accessed January 1, 2019.

5. World Health Organization. First WHO global consultation on regulatory requirements for xenotransplantation clinical trials. Changsha: The Changsha Communiqué . 2008. http://www.who.int/transplantation/xeno/ChangshaCommunique.pdf. Accessed January 1, 2019. See also Xenotransplantation 2008;16:61-63.
6. World Health Organization. Second WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials. Geneva Consultation. 2011. https://www.who.int/transplantation/xeno/report2nd_global_consultation_xtx.pdf?ua=1 . Accessed January 1, 2019.
7. Fishman JA, Scobie L, Takeuchi Y. Xenotransplantation-associated infectious risk: a WHO consultation. Xenotransplantation 2012; 19: 72-81.

Legend to the Figure

Fig. 1. Official photograph of delegates at the Third WHO Global Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials, Changsha, China, December 12-14, 2018.

First row, standing; left to right
Ying Chen, China
Yanrong Lu, China
Zhengmin He, China
Chung-Gyu Park, South-Korea
Hongjiang Wei, China
Kefeng Dou, China
Shaoping Deng, China
Eckhard Wolf, Germany
Pierre Gianello, Belgium
Richard N Pierson 3rd, USA
Emanuele Cozzi, Italy
Ralph Reinhard Tönjes, Germany
Shuji Miyagawa, Japan
Muhammad M Mohiuddin, USA
A Joseph Tector, USA
Pengzhi Hu, China

Second row, sitting; left to right
Shounan Yi, China
Rita Bottino, USA
Megan Sykes, USA
Judith (Horvath) Arcidiacono, USA
Henk-Jan Schuurman, the Netherlands
Wayne J Hawthorne, Australia
Wei Wang, China
José Ramón Nuñez, Switzerland
Léo Bühler, Switzerland
Guogang Zhang, China
Linda Scobie, UK
Peter Cowan, Australia
Curie Ahn, South-Korea
Yongfeng Liu, China
Jonathan RT Lakey, USA
Agnes Azimzadeh, USA

ABSTRACTS OF PRESENTATIONS

OPENING SESSION

OVERVIEW OF THE THIRD WHO GLOBAL CONSULTATION ON REGULATORY REQUIREMENTS FOR XENOTRANSPLANTATION CLINICAL TRIALS

Wayne John Hawthorne
Department of Surgery, Westmead Clinical School, University of Sydney
National Pancreas and Islet Transplant Laboratories, Westmead Hospital, Westmead
Centre for Transplant and Renal Research, Westmead Institute for Medical Research

INTRODUCTION: The Third WHO International Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials ("10 year review of the Changsha Communiqué") is convened once again in Changsha, China, 12-14 December 2018. The meeting commemorates a decade since the initial "Changsha Communiqué" International Consultation on Xenotransplantation was held in Changsha, China, 19-21 November 2008, and seven years since The Second WHO International Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials ("Geneva Consultation") convened at WHO headquarters in Geneva, Switzerland, on October 17-19, 2011. The Third Consultation was initiated by the International Xenotransplantation Association (IXA) with support from The Transplantation Society (TTS) together with the WHO. Invited delegates including international health regulatory authority representatives and internationally recognized experts in xenotransplantation science, technology, law, and ethics from most WHO regions, representing a substantial number of WHO member states. The charge to delegates and outcomes for the Communiqué are:

1) To review the current status of xenotransplantation science and practice;
2) To determine whether updates to the Changsha Communiqué's guidance to WHO, member state health regulatory authorities, and study investigators and/or sponsors of xenotransplant trials are required; and
3) To discuss and refine draft consensus guidelines for infectious disease surveillance, prevention, and response, appropriate to support various imminent clinical xenotransplantation trials.

The program is based on the principals and guidance documents of the previous Communiqué: working groups are created to revisit the guidelines post-Geneva. The program will critically review the Changsha Communiqué Principles and Guidance, revisiting and prioritizing essential requirements for clinical trials. In particular working parties will address the necessity to revise guidelines and policy in undertaking clinical trials. Through presentations they will formulate the changes required to the guidelines and policy in order to safeguard safety and efficacy of imminent clinical trials.

DISCUSSION: The reasoning behind the present WHO consultation is the substantial progress in the field of Xenotransplantation during the past several years, specifically in regards to the development of new technologies, donor animal genetics, donor source herd holding requirements, changing legislative frameworks (internationally), definitions and processes for submitting clinical trial applications. This is along with the emergence of a number of potential applications for Xenotransplantation trials in islet cell, cornea and kidney

transplantation. The development of the regulatory requirements for such clinical trials will be revised along with the requirements for comprehensive safety, efficacy, longevity and monitoring of the transplanted cells, tissues, or organs.
CONCLUSION AND/OR RECOMMENDATIONS: This WHO Global Consultation has the prospect to strongly reconfirm the contribution by IXA and WHO in the regulation of xenotransplantation, and to provide a revision to WHO Mémoire on minimizing risks in xenotransplantation with a realistic potential for success in pilot clinical trials in selected patients.

SESSION I - Update on progress in solid organ xenotransplantation (2012-18)

Preclinical Studies

PRECLINICAL STUDIES: ADVANCEMENTS IN CARDIAC XENOTRANSPLANTATION

Muhammad M Mohiuddin
University of Maryland School of Medicine, Baltimore, MD

INTRODUCTION AND DISCUSSION: Recently there has been a surge in reports describing fantastic survival results for solid organ xenotransplantation. Our group and others continue to show significant improvement in survival of cardiac xenografts in a heterotopic pig-to-baboon heart transplantation model. This presentation will highlight some significant advances in the field of heart xenotransplantation in large animal models. A major reason for the improved graft survival is the advancement in the technology, including CRISPR-Cas9 technique, to genetically modify the donor pigs and make their organs and tissues less immunogenic to nonhuman primates and humans. Some of the major genetic modifications will be discussed along with their specific impact on averting xenograft rejection of heterotopic cardiac xenograft. Another development that has significantly helped xenotransplantation research is the availability of target specific antibodies, to block specific immune pathways, like CD40-CD40 ligand mediated costimulation pathway, which have made a significant impact in the advancement of this field by suppressing rejection. The specific role of these antibodies in preventing xenograft rejection will also be discussed. Dr Bruno Reichart' s group from Munich, Germany, has reported significant pig cardiac xenograft graft survival in orthotopic position and some of the information presented by this group at the transplantation meetings will be briefly discussed. Other major factors that have also been very helpful are: the utilization of novel monitoring methods for the graft function and improvements in methods to manage post-operative transplant animals, especially the ability to detect complications at an early stage with the ability to overcome these adverse effects.

CONCLUSION: Finally, the impact of the above advancements in bringing xenotransplantation to the clinic and potential remaining hurdles for clinical translation will be reviewed.

PRECLINICAL STUDIES: KIDNEY AND LIVER XENOTRANSPLANTATION

Andrew Adams[1], Matthew Tector[2], Joseph Tector[2]
[1]Emory University School of Medicine, USA, [2]University of Alabama, Birmingham, USA

INTRODUCTION: Kidney xenotransplantation: The shortage of suitable donor organs has pushed the need for xenotransplantation to the forefront of transplant research. Genome editing tools for use in pigs has improved dramatically in the last 5-10 years, paving the way for the creation of new donor pigs with fewer xenoantigens, and human transgenes that can downregulate the recipient immune response. While there are a significant number of waitlisted patients with no detectable xenoreactive antibodies to new xenoantigen-deleted pigs, there is no case where a primate has a negative crossmatch. Nonhuman primate recipients with high pre-transplant donor antibody levels reject renal xenografts in less than 6 days, but multiple groups have shown prolonged kidney xenograft survival past 180 days is feasible in recipients with more favorable pre-transplant crossmatches using costimulatory blockade-based immunosuppression. Human transgenes (complement regulation and thromboregulation) are helpful in preventing early antibody-mediated rejection, but renal xenograft failure is still secondary to antibody-mediated rejection in all cases. Results using calcineurin-based immunosuppression regimens are less favorable, with graft survival rarely going past 20 days, regardless of the crossmatch status. While these results are discouraging, they are not different than the survival achieved in crossmatch-positive renal allotransplantation in non-human primates.

Liver xenotransplantation: The need for more liver donors is significant, but clinical application of liver xenotransplantation has been prevented because of the thrombocytopenia that occurs immediately following graft reperfusion. The best survival in a pig-to-nonhuman primate model of liver xenotransplantation approaches one month. The thrombocytopenia was overcome by infusing human coagulation factors. The cause of failure in the recipients going out past two weeks was aberrant clotting and thrombosis of major vasculature. There is a need for new genetic engineering strategies to overcome the thrombocytopenia, and eliminate the need for infusion of exogenous coagulation factors. The deletion of Asialoglycoprotein Receptor 1 in pigs decreases the platelet removal from ex-vivo perfusion circuits, but this strategy has not been tested in vivo in a preclinical model.

DISCUSSION AND RECOMMENDATIONS: Kidney xenotransplantation: Improving survival in preclinical models will depend upon the identification and elimination of new xenoantigens on the surface of pig cells. If a crossmatch-negative xenograft can be performed in a preclinical model, it will be possible to evaluate whether calcineurin-based immunosuppression can prevent cell-mediated xenograft rejection well enough for clinical trials.

Liver xenotransplantation: It is likely that bridging trials where a patient with significant liver failure is transplanted with a pig liver to stabilize them until a human liver can be found will be the initial clinical application for liver xenotransplantation. Significant work needs to be done using newly available pig livers in a preclinical model before moving forward with clinical application.

PRECLINICAL STUDIES: LUNG

Agnes Azimzadeh
Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

INTRODUCTION: Substantial progress has recently been observed in understanding the mechanisms of injury in lung xenotransplantation, and overall in vivo lung xenograft survival and function. Removal of the main carbohydrate antigen recognized by human anti-pig antibodies (alpha-gal) prevented the immediate destruction of pig lungs by human blood. The knockout of additional “non-Gal” carbohydrate gene targets (Neu5Gc, beta-4Gal) added incremental benefits. The addition of specific human genes (transgenes) expressed on the alpha-galactosyl transferase-deficient background demonstrated important roles for complement regulation (membrane cofactor protein, CD46), coagulation pathway dysregulation (endothelial protein C receptor, tissue factor pathway inhibitor, von Willebrand factor) and NK cells (HLA-E), as shown by improvements of specific parameters in ex vivo lung perfusion experiments. At the last IXA meeting, survival for over one week of pig lungs in baboons has been reported by two groups.

Although the lung represents a very difficult organ xenotransplant model, these results present very substantial progress, highlighting the potential of genetic modifications to prevent xenotransplant injury mechanisms. Moving forward, data from ex vivo and in vitro perfusion models identify platelet and neutrophil adhesion, monocyte/macrophage activation, glycan-based cellular adhesive interactions and specific pro-inflammatory cytokines, as logical targets for additional interventions. Future studies will address these residual injury mechanisms either through genetic modifications of the donor pig, or using pharmacologic treatments. In addition, immunosuppression or immunomodulation of the recipient will be tailored to avoid and promote immune tolerance.

PRECLINICAL STUDIES: TOLERANCE AND REGULATORY T CELLS

Megan Sykes, David Ayares, Adam Griesemer, Robert Hawley, Hao-Wei Li, David H. Sachs, Kazuhiko Yamada
Columbia University, New York, USA

INTRODUCTION: The immune and non-immune barriers to xenografts are even stronger than those to allografts. The ability to genetically engineer source animals has improved outcomes of pig-to-primate xenotransplantation, but high levels of immunosuppression are

required with many side effects, and graft rejection nevertheless eventually occurs. While the development of the αGal-knockout pig has been a major advance, studies in nonhuman primates indicate that other, non-Gal, natural antibody specificities can be targets of rejection when transplanting αGal-knockout (GalT-KO) organs. Several carbohydrate targets of anti-non-Gal natural antibodies have been identified and the genes producing enzymes that generate these epitopes have now also been knocked out. However, these additional knockouts appear to express new epitopes recognized by baboon natural antibodies.

Induction of immune tolerance, in which the recipient's immune system treats the donor as "self', eliminates responses to donor antigens, including these newly revealed determinants, avoiding the need for long-term immunosuppression while assuring long-term graft survival. Tolerance is therefore particularly desirable in the challenging setting of xenotransplantation.

DISCUSSION: Studies in rodents and humanized mice have demonstrated the efficacy of two methods of tolerance induction, namely pig thymus transplantation and induction of mixed hematopoietic chimerism. Induction of mixed xenogeneic chimerism can simultaneously achieve tolerance of natural antibody-producing B-cells recognizing known and unknown specificities, T cells and natural killer (NK) cells, all of which pose significant barriers to xenotransplantation. Moreover, mixed chimerism can be achieved with relatively non-toxic conditioning regimens that overcome the immune barriers to the xenogeneic species, which include several components of the innate immune system in addition to T cells. The use of a human CD47-transgenic source pig and intrabone injection of the bone marrow greatly enhances mixed chimerism and tolerance induction in the pig-to-baboon model, achieving promising pig kidney graft outcomes.

An additional approach to enhancing pig chimerism in the baboon model involves infusion of expanded regulatory T-cells from the recipient along with pig hematopoietic cells. This approach has enhanced pig skin graft survival on baboons. Simultaneously, our group has pursued the approach of recipient T-cell depletion combined with pig thymus transplantation. Proof of the efficacy of porcine thymic transplantation in inducing human T-cell tolerance has been obtained in humanized mouse models, in which diverse, normal human T-cells develop in porcine thymic xenografts and are tolerant to the porcine donor, the recipient mouse and the human hematopoietic stem cell donor. This approach has been extended to the pig-to-baboon combination using vascularized pig thymus grafts. Sustained life-supporting porcine kidney xenograft survival has been achieved reliably with this approach, with evidence for the development of T-cell tolerance to the donors.

CONCLUSION AND/OR RECOMMENDATIONS: Using existing genetic modifications of porcine source animals, it seems likely that tolerance and graft survival without chronic immunosuppression should be achievable in the pig-to-human combination within the near future. This approach would be optimal in view of the very strong immunologic barriers to porcine xenotransplantation. Given the time required for generation and approval of a clinical-grade source pig, efforts to obtain this status for GalT-KO, hCD47-transgenic source pigs in anticipation of clinical trials are currently justified.

PRECLINICAL STUDIES: REGULATORY ASPECTS

Henk-Jan Schuurman
SchuBiomed Consultancy BV, Utrecht, The Netherlands

INTRODUCTION: Paragraph 4 of the Changsha Communiqué (2008) states "*Because of these wider community risks, xenotransplantation clinical trials and procedures need to be effectively regulated. There should be no xenotransplantation in the absence of effective regulation by the government of the country. Regulation should have a legal basis with powers to ban unregulated procedures and enforce compliance with regulatory requirements. "The regulatory system should be transparent, must include scientific and ethical assessment and should involve the public".* At that time major regulatory documents were from USA, i.e., the Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation (2001), and the Xenotransplantation Guidance for Industry from the Food and Drug Administration (FDA, 2003, updated in 2016). Also, in New Zealand the Health Research

Council had issued Guidelines for Preparation of Applications Involving Clinical Trials of Xenotransplantation (2007); this document is noteworthy, because the first clinical trials under proper regulatory oversight were conducted in this country (porcine islets for the treatment of diabetes, approved in 2007).

Much has happened since the 2008 WHO Global Consultation meeting in Changsha. A few examples are worth mentioning. The European Medicines Agency issued in 2009 the Guideline on Xenogeneic Cell-based Medicinal Products, and the revised guidance "Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells" is presently under public consultation. In 2017 the FDA released the Draft Revised Guidance #187 “Regulation of Animals with Intentionally Altered Genomic DNA”. In Australia, goods that comprise or contain live animal cells, tissues or organs are since 2017 regulated as biologicals. In Japan new or amended jurisprudence that affect xenotransplantation products was introduced in 2013, namely the Act on the Safety of Regenerative Medicine, and the Pharmaceutical and Medical Devices Law. In South-Korea the Ministry of Health and Welfare is developing a legal frame work for xenotransplantation, which is relevant in view of prospected xenogeneic cornea trials. In China the oversight of clinical trials has assigned to the National Health and Family Planning Commission, the former Ministry of Health.

According to the general definition, xenotransplantation products encompass live organs, tissues, and cells. These products are regulated in different ways. Human donor-derived organs are not considered as medicinal products, i.e., organ transplantation is regulated differently as drugs/biologicals; on the other hand, for cell therapy - either autologous, allogeneic or xenogeneic - a large regulatory framework has been developed worldwide during the last two decades. Noteworthy, non-live xenogeneic material is not considered a xenotransplantation product in regulatory terms; this is exemplified by the designation “xenograft” in the recently updated FDA Guidance, and the regulation as a medical device by most regulatory agencies. Therefore, regulatory xenotransplantation documents focus on safety, in particular the potential cross-species transmission of infectious agents after entry in the host. To this end, the FDA Guidance has introduced a new status of pathogen presence, designated pathogen-free (DPF), a status in between specific pathogen-free (SPF) and gnotobiotic.

For the swine species, generally accepted as the donor species for xenotransplantation, the DPF status regards two major items. First, the presence of xenozoonotic exogenous infectious agents that do not affect the health status of the donor animals, but can infect and cause disease in a human recipient; e.g., herpes viruses such as gamma-lymphotropic herpesvirus, cytomegalovirus, and hepatitis E. Second, the presence of endogenous agents, i.e., Porcine Endogenous RetroVirus (PERV). Exogenous infectious agents can be eliminated from the herd by various breeding techniques in herd maintenance: in case of cell therapy products, exogenous agents can also be eliminated from the final product by various procedures during the manufacturing process starting with organ procurement and ending with cell culture. Regarding PERV, since the recognition of pig-to-human transmission in a rather artificial cell coculture condition 20 years ago, the status of present knowledge indicates that the potential of cross-species transmission is minimal and manageable. Also, gene-editing technology (such as CRISPR-Cas9) has been introduced which is claimed to inactivate the genes encoding PERV sequences.

CONCLUSION AND/OR RECOMMENDATIONS: Regulatory agencies have followed the progress in xenotransplantation research to enable the phase transition to clinical development under proper regulatory oversight. It is realized that xenotransplantation products encompass different products (organs, tissues, cells) that share microbial safety aspects, but differ in regulatory aspects addressing efficacy and functionality in development and market approval. These products also share regulatory aspects of genetically modified donors. Xenotransplantation products are based on innovative and new biomedicinal approaches, and therefore a continued dialogue between the xenotransplantation field and regulatory authorities is warranted to address risks and risk management in bringing

xenotransplantation products to clinical application in patients with end-stage organ dysfunction who are in medical need for treatment of their disease.

SESSION II - Update on progress in cell and tissue xenotransplantation (2012-18)

Preclinical Studies

PRECLINICAL STUDIES: FREE ISLETS

Rita Bottino[1], David KC Cooper[2], Massimo Trucco[1]

[1]Allegheny Health Network – Carnegie Mellon University – Pittsburgh, PA, USA

[2]University of Alabama at Birmingham, Birmingham, AL, USA

INTRODUCTION: Islet transplantation is a valuable alternative to whole pancreas transplantation to treat diabetes and reduce the life threatening hypoglycemia unawareness in patients with Type 1 diabetes. Paucity of human organ donors has led to the consideration of potential alternative sources of islets, with pig islet cells being attractive candidates for cell therapy. One of the compelling reasons for choosing the pig is the long experience in the use of porcine insulin to treat patients before recombinant human insulin became available. Porcine insulin demonstrates its biological function in humans. Validation of isolated pig islets as a viable insulin-producing tissue for transplantation has been consistently achieved in immunodeficient rodent recipients. Over the last 15 years, more rigorous animal models such as the pig-to-nonhuman primate transplantation model have been studied. Consensus has been reached within the International Xenotransplantation Association on the relevance of pre-clinical studies in nonhuman primates prior to initiating clinical trials. More specifically, proof-of-concept that porcine islets transplanted in diabetic immunosuppressed nonhuman primate recipients consistently improve metabolic control, is regarded as a key milestone to be met before human applications.

Porcine islets can be isolated from the pancreas of adult and neonatal pigs with consolidated yet challenging procedures. Islets from genetically-engineered pig donors (with tissues devoid of the alpha-Gal epitope that is target of anti-Gal natural antibodies, and transgenic expression of human factors modulating inflammation and coagulation) and islets from wild-type (non-genetically manipulated) pigs, have been successfully used in nonhuman primate recipients. Free islets (thus non-encapsulated, non-immunoisolated) are the target of innate and adaptive immunity. Immunosuppression of the recipient is therefore necessary for graft survival. Using relatively high islet doses and efficacious, yet not clinically applicable, immunosuppression, islet graft function and insulin independence have been achieved by many groups worldwide, with most recent data from Seoul National University reporting insulin-independence for more than 900 days.

DISCUSSION: Comparison of metabolic profiles in humans, nonhuman primates and pigs suggests that porcine islets may perform more efficiently in humans than in nonhuman primates. High islet doses are required to overcome the initial islet loss due to inflammation and innate immunity. Improved peritransplant management may prove critical to reduce such loss. In addition, the need for immunosuppression that is clinically applicable, thus that carries minimal additional risk to the patient than conventional anti-rejection protocols, remains an unresolved challenge. A combined effort consisting in (i) the choice of effective genetic modifications for pig donors, and (ii) selection of immunosuppressive drugs that protect the graft without increasing the risk of over- immunosuppression, may be appropriate.

CONCLUSION AND/OR RECOMMENDATIONS: Progress has been made in the pig-to-nonhuman primate models of islet transplantation. In view of clinical translation, the availability of genetically-engineered pig tissue provides a helpful tool to improve compatibility between donors and recipients. Only immunosuppressive therapy consisting of clinically applicable protocols that warrant islet survival and avoid increased morbidity to the recipients, should be considered.

PRECLINICAL STUDIES: ADVANCES IN ENCAPSULATED ISLET/DEVICE TRANSPLANTATION AS A TREATMENT FOR DIABETES

Jonathan RT Lakey
Department of Surgery and Biomedical Engineering; Clinical Islet Program, University of California Irvine, USA

Islet transplantation to treat type 1 diabetes has achieved great improvements, as more recipients are able to achieve insulin-independence for longer periods of time. Unfortunately, the lack of donor organs and immunosuppressive medication regimens continue to impede further progress in cell-replacement therapy. Encapsulation of islets for transplantation provides a solution to these problems. Cell encapsulation envelopes cells in a biocompatible matrix that provides a gradient which allows the diffusion of oxygen and nutrients but prevents large immune molecules from reaching the cell, avoiding a host immune response. Encapsulation has been suggested since the 1930's but noteworthy achievements have occurred over the last decade. This lecture aims to provide a review including a historical background, current research, and future applications of cell encapsulation for the treatment of type 1 diabetes.

Nano-encapsulation has been used to improve diffusion parameters and better islet insulin response. Polyethylene glycol (PEG) is one of the most common materials used in nano-encapsulation devices as it can crosslink under ultraviolet or visible light exposure without threatening cell viability. Nevertheless, the shortcomings of PEG include the lack of biocompatibility with the transplant recipient and inadequate protection of islets from cytokines. However, by using multi-layer PEGylation and immunosuppressive drug cocktails, islets have demonstrated increased stability and longer survival time while minimizing the immune response, as indicated by the reduction in human serum albumin, fibronectin, and IgG.

Both synthetic agents, from polyethylene oxide to polyvinyl alcohol, and naturally-occurring hydrogels, like gelatin, chitosan, and alginate, have been utilized in encapsulation engineering and in extracellular matrixes. Though polyglycolic and lactic acid polymers are some of the more popular synthetic agents in medical devices, they still pose the risk of increased fibrosis and loss of the encased cells. Nevertheless, synthetic biomaterials are still being frequently used, with PEG being the most widely used synthetic biomaterial for islet encapsulation, though different encapsulation strategies have varying levels of success. Such strategies include assembling a thin layered PEG-lipid structure around the surface of islets and assembling a multilayer film around islets using biotin and streptavidin.

Due to the complications with islet encapsulation using synthetic materials, alginate encapsulation has risen in popularity due to its improved biocompatibility and stability, easy gelation process, and relatively low cost. Alginate has typically been the most popular microencapsulation material, due to its widespread availability and ease of production, although alginate endotoxin content and purity can vary from different manufacturers. The variation in alginate production and purification, in addition to the lack of research regarding the optimal transplantation site of islets and optimal donor strain and age, currently stand in the way of consistent success in transplanting alginate-encapsulated islets into humans. In an effort to improve capsule permeability and mechanical strength, studies have used polycations and anions in the encapsulation process, although it often results in a greater host biologic response to the transplant.

PRECLINICAL STUDIES: PROPOSED CLINICAL TRIALS OF CORNEA AND PANCREATIC ISLET XENOTRANSPLANTATION

Chung-Gyu Park[1,2,3,4,5,6], Kim Kwang-Won[7], Byoung-Joon Kim[7], Jun-Seop Shin[1,2,3,5], Chang Ho Yoon[1,8,9], Mee Kum Kim[1,8,9]

1. Xenotransplantation Research Center, Seoul, Korea
2. Institute of Endemic Diseases, Seoul, Korea
3. Cancer Research Institute, Seoul, Korea
4. Biomedical Research Institute, Seoul National University Hospital, Seoul, Korea

5. Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Korea
6. Department of Biomedical Sciences, Seoul National University Graduate School, Seoul, Korea
7. Division of Endocrinology, Department of Internal Medicine, Gachon University School of Medicine, Incheon 21565, Korea
8. Laboratory Of Ocular Regenerative Medicine and Immunology, Seoul Artificial Eye Center, Seoul National University Hospital Biomedical Research Institute, Seoul, Korea
9. Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea

INTRODUCTION: Xenotransplantation using fresh porcine corneas has been suggested as a feasible alternative to overcome the shortage of human donor corneas. Porcine islet is also considered the best alternative for allogeneic human islets to treat the selected type 1 diabetes patients with severe hypoglycemic unawareness and glycemic liability despite conventional insulin therapy. In this announcement, we discuss preparations of clinical trial protocols to conduct the first corneal and islets xenotransplants. To ensure safe and transparent clinical trials, all related procedures were standardized based on the international consensus established by IXA and WHO.

DISCUSSION: Regarding corneal xenotransplantation, detailed contents of the protocol have been modified with reference to comments provided by ophthalmologists and multidisciplinary experts, including an infection specialist, an organ transplantation specialist, a clinical pharmacologist, a neuropsychiatrist, a laboratory medicine doctor, and a microbiologist. Two patients with bilateral legal corneal blindness (best-corrected visual acuity ≤20/200 in the better eye and ≤20/1000 in the candidate eye) will be enrolled. During the screening period, participants and their close contacts will have two separate deliberation periods before signing informed consent forms. Each patient will undergo corneal xenotransplantation using fresh cornea from Seoul National University (SNU) miniature pigs. Commercially available immunosuppressants will be administered, and systemic infection prophylaxis will be performed according to the program schedule. Data and safety monitoring board (DSMB) is independently organized and will monitor the protocol adherence, patient eligibility, intensity of follow-up, and regular review of the data. After transplantation, each patient will be monitored at a specialized clinic to investigate safety up to 2 years and efficacy up to 1 year.

For islet xenotransplantation, two type 1 diabetes patients who meet selection and exclusion criteria will be enrolled. Primary purpose of this pilot trial is to confirm the safety of porcine islets. Porcine islets will be isolated from SNU miniature pig and manufactured in a GMP-compliant facility and prepared to meet IXA islet product release criteria including sterility, potency, and viability. Clinically applicable immunosuppression regimen modified from the one which has been developed from pre-clinical studies in nonhuman primates will be administered to warrant the safety of the patients. Independent DSMB will examine the protocol adherence, patient eligibility, follow-up schedule, and resulting data. After transplantation, two patients will be monitored to investigate the safety of porcine islets up to 2 years and then followed by life-long surveillance according to the law that will be supposed to be implemented by Korean government.

CONCLUSION AND/OR RECOMMENDATIONS: Detailed clinical trial protocols for the first corneal and islet xenotransplants reflecting the global guidelines are provided. For a safe and transparent clinical trial, all procedures must be standardized and clinical trial protocols should be prepared.

PRECLINICAL STUDIES: HEPATOCYTES XENOTRANSPLANTATION

Leo H. Buhler
University Hospitals Geneva, Geneva, Switzerland

INTRODUCTION: The treatment of acute liver failure is based on optimal supportive medical care and, in the most severe cases, liver transplantation. The latter option represents the only treatment available in cases of end-stage liver failure. The shortage of organ donors limits its application, and the post-transplant care includes lifelong immunosuppressive

therapy. Thus, the development of alternative therapies is crucial to bring new therapeutic strategies to the clinic.

Hepatocyte allotransplantation has been proposed for temporary liver function support, while the injured liver regenerates, or while waiting for transplantation. As hepatocyte allotransplantation is also facing organ shortage, only marginal livers are directed to this approach, and the quality of isolated cells is limited. Therefore, the use of xenogeneic liver cells has been proposed using encapsulation methods to immunoisolate the implanted cells, thus avoiding post-transplant immunosuppression. The choice of using the peritoneum as implantation site allows the implantation of significantly higher volumes of cells, compared to intra-hepatic implantation.

We have therefore developed techniques for isolation of large volumes of porcine hepatocytes, obtained from freshly procured livers and microencapsulated these cells with new types of polymers based on polyethylene glycol-alginate. In a pig-to-nonhuman primate transplantation model, the encapsulated porcine hepatocytes were transplanted into baboons with acute liver failure induced by 75% liver resection and warm ischemia of the remaining liver segment. Fulminant liver failure was characterized by typical modification of liver biochemical parameters, severe steatosis, and massive hepatocyte necrosis within the first 10 days. Hepatocytes from miniature swine were microencapsulated in alginate-based microspheres, and transplanted intraperitoneally immediately after liver injury. We could show that microencapsulated porcine hepatocytes provide temporary liver function support in baboons with fulminant liver failure.

DISCUSSION: To bring this approach to clinical application, we are working on the establishment of a clean pig facility, a GMP-grade laboratory space, and plan to repeat the pig-to-nonhuman primate study with higher numbers of animals. We also aim to improve the viability and function of the isolated and encapsulated hepatocytes by co-transplanting mesenchymal stromal cells with the hepatocytes. We have contacted the Swiss Federal Regulatory Agency for Drug Administration, Swissmedic, to establish a road map for initiation of a clinical trial.

CONCLUSION AND/OR RECOMMENDATIONS: A clinical trial of porcine hepatocyte transplantation in patients with acute liver failure could be initiated in centers where human organ donors are not easily available on an emergency basis. The ethical and regulatory aspects are currently being implemented.

SESSION II - Update on GM pigs & facilities, proposed clinical trials, and microbiological testing

GENETICALLY MODIFIED DONOR PIGS FOR XENOTRANSPLANTATION – STATE OF THE ART

Eckhard Wolf[1], Bruno Reichart[2]

[1]Gene Center and [2]Walter-Brendel Center for Experimental Medicine, LMU Munich, Munich, Germany

INTRODUCTION: The use of pigs as source of cells, tissues and organs for xenotransplantation offers the unique opportunity of genetic engineering the donor animals. Gen(om)e editing is speeding progress in this field. More than 40 different genetic modifications have been introduced into pigs to prevent immune rejection of xenografts, overcome physiological incompatibilities, and reduce the risk of transmitting zoonotic pathogens. Genetic modifications to overcome hyperacute and acute vascular rejection of pig-to-primate xenografts include the inactivation of the α-1,3-galactosyltransferase (GGTA1) gene to eliminate the major xeno-antigen galactose-α1,3-galactose (αGal). Other prominent xeno-antigens, i.e. N-acetylneuraminic acid (Neu5Gc) and an Sd(a)-like glycan, have been eliminated by inactivating the genes encoding cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) and β-1,4-N-acetyl-galactosaminyl transferase 2 (B4GALNT2).

A complementary approach is transgenic expression of human complement-regulatory proteins, such as CD46, CD55 and CD59, singly or in combination. Porcine cells and tissues with lacking or reduced swine leukocyte antigens (SLA) should elicit only weak responses from the nonhuman primate/human immune system: MHC class I deficient pigs have been reported and these show reduced levels of CD4- CD8+ T cells in the peripheral blood, but appeared healthy and developed normally. Dysregulation of coagulation and disordered haemostasis are frequent complications in preclinical pig-to-nonhuman primate xenotransplantation. Key endothelial anticoagulant/antithrombotic proteins that have been modified/supplemented by genetic engineering of donor pigs include human thrombomodulin (THBD), endothelial protein C receptor (EPCR), tissue factor pathway inhibitor (TFPI), and ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1 alias CD39). In addition, transgenic pigs that express anti-apoptotic and anti-inflammatory proteins, such as human tumour necrosis factor-alpha-induced protein 3 (A20) and human heme oxygenase-1 (HO-1) have been produced.

Transgenic strategies to overcome cellular rejection of pig-to-primate xenografts include the expression of CTLA4-Ig or LEA29Y (to block co-stimulation of T cells), of HLA-E/beta2-microglobulin (to protect porcine cells against human NK-cell mediated cytotoxicity), and of human CD47 (to suppress phagocytic activity of monocytes/macrophages). Aberrant phagocytosis of human platelets during perfusion of porcine livers could be partially overcome by deleting the porcine asialoglycoprotein receptor (ASGR).

Genetic modifications which decrease the risk for porcine endogenous retrovirus infections include the long-term reduction of PERV expression via PERV-specific siRNAs and CRISPR/Cas9 mediated inactivation of PERV proviruses by mutating their pol genes.

DISCUSSION: The combination of genetic modifications required may depend on the type of organ/tissue and – especially for cellular xenografts – the transplantation site. Cellular localisation and level of transgene expression are critical for the functionality and potential side effects of specific modifications. Segregation of independent integration sites is avoided by “combineering” and “gene stacking”, i.e., random or targeted placement of multiple expression cassettes in a single genomic locus.

CONCLUSION AND/OR RECOMMENDATIONS: Technical advances in the generation of genetically multi-modified pigs and new developments in the field of immunosuppression led to significant progresses in many areas of xenotransplantation, including pancreatic islets, but also vascularised organs like kidneys, hearts and lungs. Xenotransplantations can thus be considered as realistic future therapeutic options together with regenerative medicine strategies, e.g., stem cells.

PIGS AS A XENOGRAFT SOURCE ANIMAL AND BIOSECURITY IN SOUTH KOREA

Curie Ahn
Division of Nephrology, College of Medicine, Seoul National University, Seoul, South-Korea

INTRODUCTION: For the treatment of incurable diseases, transplantation has been performed during the past several decades. However, there has been a severe shortage of transplantable organs, so that xenotransplantation research has been conducted using alternatives. Miniature pigs might be the suitable model of organ donor for xenotransplantation because of their physio-anatomical similarity to humans. In xenotransplantation research, immunological rejections and infections such as PERV, PLHV, PCMV, and other xenozoonosis infections are major barriers that need to be overcome for successful xenotransplantation. Microbiological safety is an important issue in pigs used as xenograft source. “The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet production in type I diabetes” was announced, and it contained some criteria such as designated pathogen list and standard operation procedures for source animal production. To establish a biosecure condition, all steps such as quarantine, rederivation, microbiological screening, veterinary care, individual data for medication, should be strictly controlled. Recently, several biosecure pig facilities for xenograft were successfully launched and maintained in South Korea. Especially, new infrastructure for xenotransplantation research, named designed animal resource center (DARC), started operation from 2014, and it has the capacity for 150 pigs and 200 nonhuman primates.

BARRIER FACILITIES FOR DPF SOURCE PIGS: CHINA EXPERIENCE

Wang Wei
The Cell Transplantation and Gene Therapy Institute, The Third Xiangya Hospital of Central-South University; Changsha, China

INTRODUCTION: Paragraph 2 of the Changsha Communiqué (2008) states “Animals used in xenotransplantation should be from a closed herd bred for the purpose and housed in a well-controlled, pathogen-free environment with high standards of animal welfare. Source animals should be extensively tested to ensure freedom from known pathogens with appropriate biosecurity and surveillance in place to ensure continued freedom from infectious disease.” The consensus statement published in 2009 by IXA provided guidance about barrier facility and Designed pathogen free pig.

We collected more than 10,000 samples from 11 Chinese local closed pig breeds [1], and found a pig specimen with PERV-C gene deficiency in 2002. By inbreeding 22 generations, we established stable and closed herd inbred pigs with high islet yields and we named it Xeno-1. Genomic analyses and experiments revealed that Xeno-1 is natural PERV-C free pig which could greatly reduce the potential risk of cross-species transmission of PERVs.

We established standards on Medical grade DPF donor pig for xenotransplantation, including genetic standard, microbiological surveillance, formula feeds, regulation for pathological diagnosis, requirements of environment and housing facilities, and a list of designated pathogens to be excluded from the source herd based on consensus statement published in 2009 by IXA [2]. We established a GMP barrier facility for DPF source pig in Changsha, China in 2012. This facility is inspected and approved by the third-party authorities, including National Institutes for Food and Drug Control, State Key laboratory of Virology, and CDC of Hunan Province. We verified the bio-safety of DPF source pig in humanised mouse model and nonhuman primate model. Approved by the government, we conducted a clinical trial with Type 1 diabetes patients under the regulatory framework for xenotransplantation. After 5 years follow-up, no PERV transmission was found in the patients and their wives. In addition, we also confirmed that no microchimerism happened.

DISCUSSION AND CONCLUSION: Source pigs used in xenotransplantation should have a clear and stable genetic background, so as to ensure they always be PERV-C negative and easy to breed under barrier facility. Pig with unstable genetic background is easy to be infertile under barrier facility. Well trained staff, strict and operational SOP, accurate and rigorous data

retention, are all important for the operation in GMP barrier facility for DPF source pig. GMP barrier facility and DPF source pig should be under bio-safety surveillance by authorized third party. The bio-safety of DPF source pig must be verified and confirmed by biological tests. **RECOMMENDATIONS**: DPF source pig need GMP barrier facility, SOP, regulation and bio-safety surveillance by the third-party authorized lab. Source pigs used in xenotransplantation should have a clear and stable genetic background. Develop a list of pathogens to be excluded from the source pig according to local state/region guidelines.

1. Xing X, Xu S, Mo Z, et al. Analysis of subtypes of porcine endogenous retrovirus in hybrids of Landrace with Qinghai Bamei swine. Chinese Journal of Zoonoses, 2007,23(9):878-882
2. Schuurman HJ. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes--chapter 2: Source pigs. Xenotransplantation. 2009;16:215-222.

BARRIER FACILITIES: USA

Agnes Azimzadeh

Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

INTRODUCTION: Xenotransplantation of porcine cells, tissues, and organs is associated with the potential transmission of porcine microorganisms to the human recipient. Several groups of experts throughout the world have reviewed potential infectious risks associated with xenotransplantation. The World Health Assembly was consulted in 2004 and several consultations with the World Health Organization (Geneva 1997, Changsha 2008, Geneva 2011) provided guidance documents. The IXA published several consensus statements for future clinical trials of xenotransplantation. In parallel to those efforts, the U.S. Department of Health and Human Services (DHHS) published a Public Health Service (PHS) Guideline on Infectious Disease Issues in Xenotransplantation to address the infectious disease concerns raised by xenotransplantation. The Guideline was developed within DHHS by the Center for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), Health Resources and Services Administration (HRSA), National Institutes of Health (NIH), and the Assistant Secretary for Planning and Evaluation. Published general principles provided guidance on the development, design, and implementation of clinical protocols for potential sponsors and local review bodies of xenotransplantation clinical trials, in preparing submissions to FDA or the Secretary's Advisory Committee on Xenotransplantation (SACX). Clinical trials conducted within the United States are subject to regulation by the FDA. FDA has published a guidance document ("Guidance for Industry: Precautionary Measures to Reduce the Possible Risk of Transmission of Zoonoses by Blood and Blood Products from Xenotransplantation Product Recipients and Their Contacts") (2003). All these documents provided a basis for the production of safe donor pigs for xenotransplantation. The PHS Guideline acknowledges the complexity of infectious disease issues and recommends ongoing discussions of scientific and medical issues raised by xenotransplantation.

Accordingly, recent progress in the field with respect to understanding risks by PERV and other pathogens, advancement of diagnostic capabilities and modifications of the pig genome using CRISPR-Cas9 technology, have increased the relevance for further reconsideration of potential infectious risks in xenotransplantation. A "barrier facility" by definition is highly biosecure and is recommended by guidance. A recent report described the development of a Source Animal (barrier) Facility (SAF) established in the U.S. for generating designated pathogen-free (DPF) pigs to serve as donors of viable organs, tissues, or cells for xenotransplantation into clinical patients. Another facility has been designed and is awaiting imminent construction. Such facility allows for pathogen elimination and prevention within a regulated DPF closed pig herd and for long-term breeding and production of xenotransplantation materials. Specific techniques involve caesarian derivation, colostrum deprivation, sows of specified pathogen-free (SPF) health status, and equipment sterilization protocols. To reduce the risk of prion-based diseases, feed components should be traceable and free of cattle-derived materials for multiple generations prior to donation. The operations

in such a SAF should be according to current Good Manufacturing Practices (cGMP) conditions. It is also proposed that SAF animal husbandry conditions should follow those for research animals per the Guide for the Care and Use of Laboratory Animals with research facility registration and accreditation by the United States Department of Agriculture (USDA, in the USA), the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) respectively, and conduct of experiments under oversight of an Institutional Animal Care and Use Committee (IACUC).

XENOTRANSPLANTATION – PREVENTING ASSOCIATED INFECTIOUS RISK: A WHO CONSULTATION PART II

Linda Scobie
Department of Biological and Biomedical Sciences, School of Health and Life Sciences, Glasgow
Caledonian University, Glasgow, Scotland, U.K.

INTRODUCTION: In 2011, the second global Consultation on Regulatory Requirements for Xenotransplantation clinical trials was held in Geneva, Switzerland. As a result of this meeting, the manuscript "*Xenotransplantation-associated infectious risk: a WHO consultation*" was published in Xenotransplantation in 2012 [1]. The document summarised the approaches to disease surveillance in individual recipients of non-human tissues and described some general concepts. At this point, progress to the clinic was still slow and consensus guidelines and recommendations didn't always provide clear information on how to proceed. In August 2018, Xenotransplantation published a special issue on safety and state of the art prevention of transmission of porcine viruses. In addition, the FDA has updated their Guideline on Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans [2] as have the original consensus statements put forward by IXA [3,4]. In support, there have been many publications looking at sensitive assays [5] and approaches for comprehensive pathogen testing [6]. Indeed, some recent literature suggests promise for the deletion of PERV elements in pigs, if this is required [7]. The field has clearly moved forward, and as such, we are now in a position to progress to the clinic as we have much more safety data available to answer some of the previous questions posed and guideline recommendations.

The aim of the previous meeting was to bring harmonisation of global practices of Xenotransplantation, however we still do not have fully evaluated commercial assays for certain pathogens. In addition, the global inventory of xenotransplantation doesn't appear to have been updated since 2015 http://www.humanxenotransplant.org/home/ possibly due to a lack of awareness from individuals proposing trials. Questions are still being asked as to the relevance of PERV as a pathogen and what the risk would be with regard to unknown elements not detected in the initial screening of both animal and product. These are difficult to answer; in essence, to fully evaluate the microbiological risk, clinical trials are needed.

3. Fishman, J.A., L. Scobie, and Y. Takeuchi, Xenotransplantation-associated infectious risk: a WHO consultation. Xenotransplantation, 2012. 19(2): p. 72-81.
4. Food and Drug Administration. Guidance for industry: source animal, product, preclinical, and clinical issues concerning the use of xenotransplantation products in humans. 2003, updated 2016. https://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/xenotransplantation/ucm533036.pdf. Accessed June 13, 2017. 2016.
5. Spizzo, T., et al., First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes--Chapter 2a: source pigs--preventing xenozoonoses. Xenotransplantation, 2016.23(1): p. 25-31.
6. Denner, J., et al., First update of the International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes--Chapter 5: recipient monitoring and response plan for preventing disease transmission. Xenotransplantation, 2016. 23(1): p. 53-9.

7. Denner, J., Sensitive methods and improved screening strategies are needed for the detection of pig viruses. Xenotransplantation, 2017.24(3).
8. Gazda, L.S., et al., A comprehensive microbiological safety approach for agarose encapsulated porcine islets intended for clinical trials. Xenotransplantation, 2016. 23(6): p. 444-463.
9. Niu, D., et al., Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science, 2017. 357(6357): p. 1303-1307.

SESSION IV - The regulatory environment for xenotransplantation clinical trials

FDA CONSIDERATIONS ON THE INITIATION OF XENOTRANSPLANTATION CLINICAL TRIALS

Judith Arcidiacono
U.S. Food and Drug Administration

INTRODUCTION: The development of U.S. policy on xenotransplantation as a means to circumvent the shortage of human organs for transplantation into humans began around 25 years ago and continues to evolve as new technologies bring possibility of xenotransplantation closer to the clinic. In 2016, the FDA Center for Biologics Evaluation and Research (CBER) published a revision to the 2003 "Guidance for Industry: Source Animals, Product, Preclinical and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans". The primary focus of the update was to include FDA guidance documents applicable to xenotransplantation that did not exist in 2003. Currently, the FDA Center for Veterinary Medicine (CVM) is finalizing the "Draft Guidance for Industry: Regulation of Intentionally Altered Genomic DNA in Animals". This guidance document is applicable to xenotransplantation products that utilize "animals whose genomes have been intentionally altered to produce xenotransplantation products" and outlines regulatory requirements for the intentionally altered genomic DNA in such animals. The regulatory approaches utilized by both CBER and CVM are focused on reducing the potential risks for use of xenotransplantation products.

DISCUSSION: Transmission of known and unknown pathogens to recipients of xenotransplantation products is one of the primary concerns when initiating first-in-human (FIH) xenotransplantation clinical trials. A product development program for xenotransplantation products begins with the establishment of a closed source herd that has been adequately tested for adventitious agents. Programs for monitoring animal health and animal husbandry should focus on excluding infectious diseases from the pool of organ/cell donor animals. Molecular characterization of source animals that have been genetically altered should be conducted and include genotypic and phenotypic analyses.

The product development program also includes establishing protocols for the harvest of cells, tissues and/or organs from the animal and transportation to the clinical site/surgical suite. Procedures should be carried out in a facility that prevents the introduction of infectious agents. As with other biologics regulated by FDA, the xenotransplantation product must meet lot release criteria that includes identity, potency (or activity), and safety (e.g., microbiological sterility).

Prior to the initiation of FIH clinical trials, proof of concept and safety studies utilizing an appropriate animal model of disease should be conducted. Many factors should be taken into consideration in such studies, including the selection of an appropriate animal model, use of the intended clinical product, the immunosuppression regimen, the use of an immuno-isolatory device (e.g., encapsulation) when appropriate, and the need for re-implantation.

For the U.S. FDA, decisions to permit FIH clinical trials are based on established regulatory principles for cell therapy products. Clinical protocols should take into account the potential for prolonged biological activity and associated safety requirements. Recommended clinical follow-up for xenotransplantation products is 50 years. In addition to safety, a range of clinical and pharmacodynamic endpoints should be collected to inform the design of later clinical trials.

CONCLUSION AND/OR RECOMMENDATIONS: An appropriate risk/benefit profile for each xeno product must be defined prior to initiating clinical trials. The use of best practices and validated technologies as well as vigilant disease monitoring can reduce the risk of zoonotic infections. Appropriate record keeping and archiving of animal and patient samples also contribute to the safety of xenotransplantation products.

MONITORING AND CONTROL OF VIRAL INFECTIONS IN XENOTRANSPLANTATION RECIPIENTS

Minhua Luo
State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences

INTRODUCTION: Solid organ or cell transplantation is the most efficient way to treat patients with terminal organ failure. However, lack or limited organs have hampered the treatment, and pancreas is in an extremely desperate wanted status. Because of the limited resource of organs, long waiting lists and severe status of patients, plus the incomplete screening of pathogens, lethal infections happen as reported in 2003 and 2005. Two LCMV infected donors did not show clear infectious symptoms, and their organs were transplanted to several recipients who demonstrated severe infectious disease. These cases released two important messages: limited donor (donor issue) and risk of infection (safety issue).

DISCUSSION AND CONCLUSION: (1) Solution for donor issues; xenotransplantation and/or stem cell transplantation: (2) Safety issues; biosafety, virus infection, screening and monitoring virus infection

RECOMMENDATIONS: (1) Screening potential viruses from the donor (piglet) to make sure it is virus infection risk free: (2) check the infection or infection status in the potential recipients: (3) monitoring the virus infection in the recipients (the infection can from endogenous latent pathogens, acquired from the community, or the donor).

EU GUIDELINES AND POLICY TO UNDERTAKE CLINICAL TRIALS

Ralf Reinhard Tönjes
Paul-Ehrlich-Institut (PEI), Federal Institute for Vaccines and Biomedicines, Division of Medical Biotechnology, Section 6/4 Non-vital Tissue Preparations and Xenogeneic Cell-Therapeutics, Langen, Germany

INTRODUCTION: Since 2004, clinical trials of medicinal products have to be approved in Europe. Authorization of clinical trials is necessary in addition to the favorable opinion of the concerned ethics committee. This procedure has undoubtedly resulted in larger expenditures with respect to time and costs of planning and conducting of clinical trials. However, the implementation of Good Clinical Practice has increased both safety for the trial subjects and validity of the data.

In 2014, the European Parliament and Council adopted the new Clinical Trial Regulation (EU) No 536/2014, with the hope of increasing the number of clinical trials conducted in the EU. Most likely in 2020, the Regulation will replace the current European Clinical Trial Directive 2001/20/EC. It offers new principles and provisions that aim to counteract the limitations of working with the Directive, promote better harmonization and increase transparency in the reporting of clinical trial results. The assessment of a clinical trial application (CTA) will proceed through a twofold procedure based on defined timelines, with the Reporting Member State coordinating the assessment of the scientific features of the trial (part 1), and each Concerned Member State carrying out a separate assessment covering national features (part 2). In Germany, the National Competent Authorities (NCA) *Bundesinstitut für Arzneimittel und Medizinprodukte*(BfArM, Federal Institute for Drugs and Medical Devices) and *Paul-Ehrlich-Institut* (PEI, Federal Institute for Vaccines and Biomedicines) are responsible.

For advanced therapies, the same basic principles for assessment apply as for any other biotechnological medicinal product. Nevertheless, the extent of data for quality, safety, and efficacy may be highly specific. Since 2007, advanced therapy medicinal products (ATMP) including gene therapy medicinal products, somatic cell therapy medicinal products as well as tissue-engineered products, are uniformly regulated across Europe. According to Regulation (EC) No 1394/2007 tissue engineered products may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, biomolecules, biomaterials, chemical substances, scaffolds or matrices. However, products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or

metabolic action, are excluded from this definition. The Committee for Advanced Therapies (CAT) at the European Medicines Agency (EMA) has been established to meet the scientific and regulatory challenges with advanced therapies. The CAT plays a central role in the regulatory process.
DISCUSSION: The legislative framework, definitions, the procedures for submitting a clinical trial application (CTA) and an overview on the regulatory requirements for clinical trials will be provided.
CONCLUSION AND/OR RECOMMENDATIONS: Xenogeneic cell therapy is regulated as a cell-based medicinal product, for which regulations and directives exist. So far, no xenogeneic cell therapy product has achieved regulatory approval in Europe. In the long run, when the results of pig organ xenotransplantation in nonhuman primates suggest a realistic potential for success of a pilot clinical trial, highly-selected patients should be offered participation.

SESSION V - The role of IXA and WHO in the regulation of xenotransplantation

SUMMARY OF RECENT IXA-FDA MEETINGS [BOSTON 2016; BALTIMORE 2017]
ROLE OF THE IXA ETHICS COMMITTEE

Richard N. Pierson III
Massachusetts General Hospital; Harvard Medical School

ABSTRACT: Since 2015, the IXA has proactively engaged the US Food and Drug Administration (FDA) to consult regarding how new developments in the field of xenotransplantation may affect design, conduct, and regulatory oversight of clinical xenotransplantation trials. As reported recently in Xenotransplantation (1), IXA Council members and FDA representatives met informally at the 2016 American Transplant Congress meeting. Consequently, FDA and IXA jointly organized a public symposium preceding the IXA 2017 Congress. Improving preclinical organ and cell xenotransplantation outcomes were discussed in the context of previously published preclinical benchmarks. Advances in gene editing technology have enabled rapid generation of a huge variety of changes to the pig. Some combination of gene modifications to reduce immunogenicity and/or address species-specific molecular incompatibilities may simplify the immunosuppressive regimen needed to prevent innate and adaptive immune injury, and facilitate clinical translation of organ, tissue, or cell xenografts. The infectious risks associated with allo- and xeno-transplantation were compared, along with how xenozoonoses might be diagnosed and managed, should they occur. Current definitions of what constitutes a “clean” donor pig, diagnosis of unknowns pathogens, long-term archiving, and risk management for experimental subjects and their close contacts were discussed. Existing assays appear sensitive to diagnose known and unknown infectious risks; therapies likely to effectively treat infections by known organisms are available; and, ultimately, infectious safety risk can only be determined in clinical trials. The consensus was expressed that progress in the field has rendered the risk/benefit ratio more favorable for both the individual study subject and for society.

FDA presented the 2016 update of their guidance document “Source Animal, Product, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans”. FDA emphasized the role of balanced risk assessment, and a “layered” approach to optimizing subject safety, including application of best practices and ongoing process validation, as well as the importance of measuring ‘activity’ of the ‘product’ (therapeutic effectiveness), and predicting consistent performance. Preclinical “proof-of-concept” data recommendations, the possible role of initial safety or dose escalation studies, and the need for contemporaneous or historical reference groups (‘controls’) were discussed.

In the general discussion, while emphasizing that every proposal must be considered individually, consensus was explored regarding results that IXA and FDA might consider ‘sufficient’ to justify proposing a clinical organ xenograft trial in various scenarios. FDA indicated that a multigene pig and experimental treatment regimens (involving agents not-yet-approved for other uses in humans) could be considered within a single application. FDA strongly encouraged regular exchange of information and dissemination of regulatory best practices, in support of a shared FDA-IXA goal: advancing and protecting public health through the development of safe, effective xenotransplantation products.

Working in concert with TTS and in relationship with WHO, IXA’s mission is to 1) foster the science of xenotransplantation; 2) educate health care providers and lay persons; and 3) guide the development of scientifically sound, internationally consistent public policy. Advisory to the IXA Council, the IXA Ethics Committee promotes evidence-based ethical guidelines for xenotransplantation clinical trials for consideration by national regulatory authorities, trial proposers and sponsors, and any other concerned parties.

1. Cooper DKC, Cowan P, Fishman JA, Hering BJ, Mohiuddin MM, Pierson RN 3rd, Sachs DH, Schuurman H-J, Tector JA, Tönjes RR. Joint FDA-IXA Symposium, September 20, 2017. Xenotransplantation. 2017;24:e12365. https://doi.org/10.1111/xen.12365.

SESSION VI - Review of Changsha Communiqué Principles and Guidance

RECOMMENDATIONS OF THE 2nd WHO GLOBAL CONSULATION (GENEVA 2011) – HOW HAVE WE MOVED ON

Emanuele Cozzi
Transplant Immunology Unit, Padua University Hospital, Padua, Italy

INTRODUCTION. In January 2011, the Second WHO International Consultation on Regulatory Requirements for Xenotransplantation Clinical Trials took place at WHO headquarters in Geneva, Switzerland. The objectives of such a Consultation were to review the current status of xenotransplantation science and practice; to determine whether updates to the Changsha Communiqué's guidance to WHO, Member State health regulatory authorities, and study investigators and/or sponsors of xenotransplantation trials were required; and to discuss and refine draft guidance for infectious disease surveillance, prevention, and response appropriate to support various probable clinical xenotransplantation trial scenarios.

At the conclusion of the consultation, participants recommended to WHO to facilitate global collaboration for laboratory investigations; to encourage transparency in xenotransplantation related activities; and to convene regular global consultations on xenotransplantation activities. Important recommendations were also issued to the attention of Member States, investigators, proposers, or study sponsors. In particular, it was recommended that such parties should seek global consistency in requirements for clinical trials by referring to best global standards and experts' advice for issues regarding source donor animals; recipients, family members and close contacts surveillance using state-of-the-art diagnostic methodology; risk/benefit analysis; and trial infrastructure. Furthermore, it was also recommended that stakeholders should combat unfounded assertions on human xenotransplantation; and to refer to experienced independent laboratories.

At this stage, it is undisputable that a refinement or integration of existing regulatory instruments to allow improved xenotransplantation practices has taken place recently or is underway in many countries. Indeed, important regulatory changes have occurred or are in progress in several geographic areas that include Europe, Korea, Japan, and China. Such significant changes in the regulatory frameworks encompass the most diverse aspects related to the clinical application of xenotransplantation procedures and comprise ethical issues, source animals and product specifications, study monitoring, sample archiving, patient follow- up, and even insurance coverage in some legislations.

Together such measures are expected to provide a better care and protection to xenograft recipients and their close contacts, but also a higher safety profile to xenotransplantation procedures, with an ultimate net gain in terms of international public health. In this context, it is undeniable that a continued close collaboration and synergy between WHO and IXA will be of paramount importance to allow the development of a safe and successful application of clinical xenotransplantation.